MEDICAL LIBRARY

VOLUME 2 JANUARY, 1932 NUMBER 1

American Journal of Clinical Pathology

OFFICIAL PUBLICATION
THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

CONTENTS

Relations and Functions of the Clinical Pathologist in the Hospital Staff. DAVID RIESMAN 1

Prophylactic Vaccination Against Tularemia. LEE FOSHAY 7

The Experimental Production of Agranulocytosis. ROY R. KRACKE 11

A Simple Medium for the Isolation of Bacteria of the Typhoid and Dysentery Groups. JAMES C. SMALL AND WILLIAM A. KREIDLER 31

A Precipitin Test for the Diagnosis of Meningitis. WILLIAM A. KREIDLER AND MARGARET E. MURPHY 33

An Analysis of 1535 Autopsies. OSBORNE ALLEN BRINES 37

Focal Cyclic Growth as a Factor in Production of Nodular Goiter. B. MARKOWITZ 57

Modified Silver Stain for Treponema Pallidum. ELENA DE GALANTHA 63

Editorial 65

News and Notices 67

Book Reviews 69

PUBLISHED BI-MONTHLY BY THE WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.
Made in United States of America

American Journal of Clinical Pathology

EDITOR

T. B. Magath, Mayo Clinic, Rochester, Minnesota

ADVISORY EDITORIAL BOARD

C. S. Butler, U. S. Naval Medical School, Washington, D. C.

H. J. Corper, National Jewish Hospital, Denver, Colo.

B. C. Crowell, American College of Surgeons, Chicago, Ill.

Herbert Fox, Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Phila., Pa.

A. S. Giordano, 604 North Main Street, South Bend, Indiana.

F. W. Hartman, Henry Ford Hospital, Detroit, Mich.

R. A. Kelly, U. S. Veterans' Bureau, Washington, D. C.

R. A. Kilduffe, Atlantic City Hospital, Atlantic City, N. J.

J. A. Kolmer, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

K. M. Lynch, Medical College of the State of S. C., Charleston, S. C.

S. P. Reimann, Lankenau Hospital, Phila., Pa.

A. H. Sanford, Mayo Clinic, Rochester, Minnesota.

W. S. Thomas, Clifton Springs Sanitorium, Clifton Springs, N. Y.

American Society of Clinical Pathologists

OFFICERS

President, H. J. Corper, Denver, Colorado.

Vice-President, C. J. Bucher, Philadelphia, Pennsylvania.

President-elect, W. M. Simpson, Dayton, Ohio.

Secretary-Treasurer, A. S. Giordano, South Bend, Indiana.

STANDING COMMITTEES

EXECUTIVE COMMITTEE

K. M. Lynch, Chairman, Charleston, South Carolina.

F. W. Hartman Detroit, Michigan.

J. H. Black, Dallas, Texas.

F. E. Sondern, New York City.

C. E. Roderick, Battle Creek, Michigan.

A. G. Foord, Pasadena, California.

BOARD OF CENSORS

F. H. Lamb, Chairman, Davenport, Iowa.

W. T. Vaughan, Richmond, Virginia.

W. E. King, Detroit, Mich.

C. W. Maynard, Pueblo, Colorado.

Nathan Rosenthal, New York City.

F. M. Johns, New Orleans, Louisiana.

BOARD OF REGISTRY

Philip Hillkowitz, Chairman, Denver, Colorado.

Kano Ikeda, St. Paul, Minnesota.

M. W. Lyon, South Bend, Indiana.

R. W. Hammack, Los Angeles, California.

A. S. Giordano, South Bend, Indiana.

W. E. King, Detroit, Mich.

A. Yaguda, Newark, N. J.

Entered as second-class matter, January 7, 1931, at the Post-Office, at Baltimore, Md., under the act of March 3, 1879

Copyright 1932, The Williams & Wilkins Company

Medical
Direct
3-20-34

RELATIONS AND FUNCTIONS OF THE CLINICAL PATHOLOGIST IN THE HOSPITAL STAFF*

DAVID RIESMAN

Philadelphia, Pennsylvania

The honor you have done me in asking me to speak to you tonight on the occasion of your Annual Banquet I should like to requite in a becoming manner. I might perhaps do so by glorifying the clinical pathologist as he is but that would hardly be worthy of your intelligence even if it were in keeping with my own sense of scientific honesty. For the clinical pathologist has not yet attained that rank, that degree of perfection of which he is capable; therefore I shall try to portray him as of the future, to picture him as some day he will be. That method will inferentially bring out his present shortcomings.

The clinical pathologist is a product of specialization in medicine. Most of us are of two minds regarding specialization—we see advantages and we see disadvantages in its development. That it is a phase of the evolutionary process in medicine cannot be doubted and as such is as inescapable and as inevitable as the machine in industry. Its drawbacks to medicine are that it separates the profession into an ever increasing number of water-tight compartments. The day of the universal scholar in medicine—of the Charcots, the Strümpells, the Jonathan Hutchinsons, is past and as unlikely to come back as that of the universal geniuses, the Michelangelos, the Da Vincis, the Goethes. Prophecy however is always dangerous and perhaps I should not go so far as to say that such repetition of history is impossible, but scanning the world's horizon today fails to reveal the universal man either in the humanities or in the sciences, or for that matter, in statesmanship.

*Read at the Annual Banquet of the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

We must therefore content ourselves with specialism and take advantage of its virtues, which are many. The greatest is that it provides the world with men of superior knowledge in a limited field. Such specialization has been of enormous value in medicine—witness the high development of neurosurgery, urology, bronchoscopy, ophthalmology, roentgenology. What a comfort and satisfaction it is to be able to appeal to men highly trained in these special branches for help and advice.

In a hospital with its complexity of cases any one of these and other specialties may on occasion be needed but there is one which is always needed by all departments, and that is the one which you gentlemen represent—that of the clinical pathologist, and by that token your dignity rises and your responsibilities increase.

Perhaps however Dr. Lynch is right in thinking that we may be asking too much of the clinical pathologist who has to be anatomist, histologist, immunologist, chemist and bacteriologist, but the very fact that so much is asked of him is in itself a compliment.

So much depends today upon the laboratory—Dr. Fishbein has said that a + or a − may determine life or death—that the position of the clinical pathologist in the hospital is well-nigh supreme. He is called upon to aid in the interpretation of symptoms, in the diagnosis of obscure cases and in the treatment of many diseases.

In order to discharge his multitudinous responsibilities and to make use of his new-born opportunities it is of the greatest importance for him to keep in touch with clinical medicine. The test-tube pathologist cannot fulfill the function that pertains to your position.

There are a number of ways in which contact with clinical medicine may be maintained:

1. By occasional visits to the wards to see interesting cases. The clinical pathologist should of his own initiative make such visits; at the same time the members of the staff should encourage him and welcome him on the wards.

2. By a discussion of the clinical aspects of cases coming to autopsy.

3. By conducting jointly with the other members of the staff clinical and clinicopathological conferences. By the former I mean conferences on living patients either in the wards or in small side rooms, conferences attended by interns, staff and students. Not only are such exercises of enormous pedagogic value for those who attend them but they bring the clinic and the laboratory together in an ideally helpful way.

4. By active participation in the work of an intramural medical society.

All these measures help to promote contact and coöperation between the clinic and the laboratory which it will be agreed is one of the greatest desiderata in medicine. It will be easier in the future when clinicians have a sounder and broader training in the fundamental branches.

On his recent visit to Philadelphia Prof. v. Gröer of the University of Lemberg told me that before engaging in pediatrics as a specialty, he spent five years in the fundamental branches of biochemistry, physics, physiology and immunology.

Under an ideal arrangement the clinical pathologist is a full-time attaché of the hospital. That status need not prevent his answering an occasional outside call in consultation or doing a limited amount of laboratory work for the medical profession. Such activities however ought not to interfere with his primary duties to the hospital and to the staff.

These duties I should now like to elaborate a little more fully.

First of all it is, I think, important that the clinical pathologist create enthusiasm among the medical personnel of the hospital for autopsies. Although the chiefs in the wards have a similar duty, the pathologist can do a great deal in this direction by making autopsies interesting. In my days as resident physician and afterwards as pathologist the autopsy room was always filled with interns who wanted to learn. I realize that morbid anatomy is no longer the drawing card it was a generation ago. For this waning interest, which I consider most regrettable, the pathologist is largely to blame.

I do not believe that the clinical pathologist can ever achieve a really high position unless in addition to being a chemist and immunologist, he is a good morbid anatomist.

It should be remembered that for nearly a hundred years the actual leaders in medicine have nearly always been pathologists in the anatomic sense—Virchow, Rokitansky, Orth, Prudden, Welch, Councilman, Aschoff, Hektoen, Flexner, Warthin. Such men are the court of last appeal in the diagnosis of tissue changes. No matter how far chemistry and immunology may go, a profound knowledge of morbid anatomy and histology will command the highest respect and will single out the man who possesses it.

Another duty of the clinical pathologist is to teach the interns the principal tests and laboratory procedures needed in the clinic. In order that this be done properly the pathologist must be interested in teaching. He must be able to select the essentials and must have the interns for an adequate length of time. Four months is, I take it, the minimum.

He should however not limit his teaching to the interns. The senior staff in most hospitals stands also in need of instruction. In every institution there are men who deride the laboratory, although they are usually the ones who make the most indiscriminate use of it. Such men must be tactfully educated to a knowledge of what the laboratory can do and what it cannot do, and that it is economically wasteful and scientifically irrational to have all manner of tests done routinely.

On the other hand the clinical pathologist must guard against overemphasizing the importance of the laboratory, else the interns will go out with such blind faith in laboratory methods that their confidence in the time-honored, never antiquated procedures of history taking and physical diagnosis is undermined. Only the man who knows something of clinical medicine can steer safely between Scylla and Charybdis.

The pathologist should stimulate the research spirit among the interns and should have no end of problems for assignment. If these problems can be different phases of the same major research, so much the better.

I was once in the laboratory of Professor Emil Fischer in Berlin. If I remember correctly there were 167 places in one enormous room, all occupied by men working on different problems in biochemistry under the unifying influence of the great master.

I shall never forget my attendance at a so-called colloquium at which various men presented the results of their work.

That the report from the clinical laboratory should be reliable and prompt is a sine qua non although not yet attained in all hospitals. But no matter how reliable the reports may be the clinical pathologist must not be offended if the clinician does not accept them in blind faith. Laboratory reports must be judged in connection with the clinical findings.

Only recently I had a case in point. We had obtained some fluid from the chest of a patient and had sent it to the laboratory. A dogmatic report came back "endothelioma of the pleura." The physical examination and clinical history pointed to long standing cardiac disease and not to tumor of the pleura. The autopsy confirmed the clinical diagnosis and revealed no pleural tumor.

If there are enough interns to organize an intramural medical society, the clinical pathologist should be a leader in the organization and should give to the meetings a scientific tone.

He is in the best position to create a true esprit de corps, not only among the interns but among the staff as a whole. Such a spirit soon makes itself felt beyond the walls of the hospital and becomes one of the chief determinants for medical students in choosing that hospital for their internship.

A pendant to the medical society of the hospital is the library. As his department will undoubtedly make the most use of it, the clinical pathologist has the deepest interest in its maintenance and expansion.

The clinical pathologist in a small hospital in a town or city not having a medical school and far away from a medical center has a splendid opportunity of making his hospital a school of post-graduate study for interns and staff.

Under his inspiration such a hospital may become a nucleus from which a scientific spirit irradiates the entire medical profession of the community. This has been well pointed out by Funk.

The growing employment of technicians in hospitals and by physicians in private practice makes it desirable that there should be schools for this modern female species. I see no objection to

such a school being attached to the Department of Clinical Pathology in a large hospital, provided that it is conducted properly and with due regard to the primary needs of the hospital itself.

And now I come to a matter that is not strictly scientific and yet I dare not neglect to speak of it, and that is emolument. The laborer is worthy of his hire. The clinical pathologist considering his responsibilities, his devotion to an intramural job that takes him out of the current of life, ought to be well compensated, although not too well. I say not too well for the same reason that makes me differ from President Hutchins of the University of Chicago, who not so long ago stated that a professor should receive at least $50,000 a year salary. A clinical pathologist or a professor receiving such a magnificent wage would soon be bothered by his investments and in a few years might be so rich that his wife would insist on his retirement from active duty.

There is one danger connected with the clinical pathologist's compensation, that is the danger of commercialism which the whole profession faces in this era of economic upheaval. The danger must be combated on the basis of the high ethical ideals that are the tradition and the pride of medicine.

I have sketched the ideal clinical pathologist as I conceive him. To achieve the state of distinction I have in mind be must overcome any inferiority complex; he must feel himself the equal of his colleagues on the clinical side and not merely a super-technician. Then he will have that flattering and responsible position for which the time seems to be ripe—namely the position of consultant-general.

PROPHYLACTIC VACCINATION AGAINST TULAREMIA

LEE FOSHAY

From the Christ Hospital Institute for Medical Research, Cincinnati, Ohio

Several writers have commented upon the high incidence of infection among laboratory workers engaged in research on tularemia, particularly among those who handle and perform autopsies on infected animals. Serious warnings have been given to those engaged in or about to engage in such work. That the import of these warnings is based on considerable experience is attested by the reports of Francis, Ledingham and Simpson. It has been stated that no one who has autopsied animals for four or five consecutive months has escaped infection. It has further been stated that no vaccine or serum has been proved to have any protective value but I have been unable to find any records of experiments bearing on this point.

Because of the seriousness of the situation for the laboratory worker and the general belief that prophylactic measures are useless I wish to report my own experience with prophylactic vaccination during a laboratory investigation of tularemia of eight months duration. During this period more than fifty animals, chiefly guinea-pigs, have been infected and autopsied. Cultures of *Pasteurella tularensis* (*Bacterium tularense*) have been made on various mediums and vaccines and suspensions have been made in many different ways. During this period the writer has had no illnesses. At the time of writing animal experiments are still being carried on and vaccination is being continued. It is regretted that this experience is limited to only one individual.

On October 20, 1930 a guinea-pig was infected with *P. tularensis* subcutaneously with pus from the finger lesion of a patient in the General Hospital two days before her death from tularemia. Since that time the strain has been kept alive on coagulated egg yolk and cystine-ascites-agar mediums with frequent passages

through guinea-pigs. Suspensions of the bacteria have been made by washing the growth from cystine-ascites-agar slants with either 1.0 per cent formaldehyde or sterile salt solution. Because of Wherry's previous experience that heat-killed suspensions were very toxic, other methods were tried to obtain an antigen which would be available for immunization, that is one that could be given in adequate dosage without untoward symptoms and yet be sufficiently antigenic to produce immunity.

Such a vaccine was finally made by treating a heavy suspension of the bacteria with 1.0 per cent formaldehyde for twenty-four hours, then with 3 per cent hydrogen peroxide for forty-eight

TABLE 1

THE RELATION OF AGGLUTINATION TITER TO ELAPSED TIME DURING THE PERIOD OF CONTINUED VACCINATION

SERUM DILUTIONS	NOVEMBER 14	JANUARY 8	FEBRUARY 2	APRIL 1	JUNE 1
1:640	0	0	0	0	0
1:320	0	0	0	0	+
1:160	0	0	0	+	+
1:80	0	0	0	+	+
1:40	0	0	+	+	+
1:20	0	+	+	+	+
1:10	0	+	+	+	+

0 = no agglutination; + = positive agglutination.

hours and finally with 1.0 per cent sodium rincinoleate for twenty-four hours, washing the bacteria thoroughly between each chemical treatment, and finally resuspending in salt solution.

Beginning on November 14, 1930 with a minute amount of a dilute suspension of this antigen, and with no agglutinins for *P. tularensis* in my serum, I have vaccinated myself almost daily with this, and later with various other and denser antigens, gradually increasing the dose whenever that of the previous day failed to give more than a slight local reaction, a simple erythema not larger than a centimeter in diameter. Considerable care was taken not to produce constitutional symptoms. However the first eight subcutaneous injections produced a moderate amount

of general malaise, beginning about six hours after injection and lasting about four hours.

During the next three weeks only occasional general symptoms were noted, occurring only at times when the dosage was increased. After the first month no general symptoms occurred. At no time were any of the reactions very disturbing. The table shows the gradual rise of agglutinins in the serum as the vaccination was continued.

It is believed that the vaccination has afforded some protection against infection. The animal autopsies were all performed with ungloved hands and in ordinary clothes. The animals were immersed in a 1:1000 solution of mercuric chloride for ten minutes before they were tacked to the board. After completion of the examination, removal of organs, et cetra, all instruments, utensils and discarded remnants of organs were boiled. The cadaver was boiled, the board sponged with bichloride solution and the hands washed with soap and cold water. This is a reasonably careful technic but not more careful than that of other men who have become infected.

Early in May an accident caused several drops of a fresh heavy saline suspension of living organisms to fall on one wrist and run along the arm to the elbow. It was not possible to free the hands until after the liquid had dried when the arm was washed with soap and water followed by alcohol. No evidences of infection have appeared. It may be contended perhaps that it has been simply good luck, both in regard to the long period of performing autopsies and to the contamination of the skin, but certainly the weight of recorded human experience is against it. Without making any undue claims it has at least been demonstrated that the vaccine has produced a steadily increasing agglutination titer in the serum of the recipient.

Before the vaccination was begun intradermal tests were negative. During the first month of administration intradermal tests gave some erythema and itching, indicating probably that some degree of sensitivity had been acquired. Since that time several intradermal tests have been made, some with large amounts of antigen and all with wholly negative results. This is

distinctly contrary to my experience with fifteen patients who had tularemia, all of whom were extremely skin sensitive to minute amounts of antigen for at least seven months after infection.

The presumed immunity has not been put to the final test of infection with living organisms but it is thought that sufficient evidence has been adduced to indicate that probably a certain degree of it has been acquired. The method is as rational in theory as prophylactic typhoid inoculation and it is offered with the hope that others who intend to work with the disease will give it a trial. It is not practicable for the future rabbit hunter but I do think it will protect the laboratory workers.

SUMMARY

The experience of one individual with prophylactic vaccination against tularemia is presented and discussed.

A method for making an adequate, relatively non-toxic antigen is described.

It is believed that prophylactic vaccination will effectively protect man against laboratory infections of tularemia.

Note: Since this report was written I have begun to vaccinate another man who has been allowed to come into close and frequent contact with infected animals. So far he has remained well during a period of exposure of more than two months. A new vaccine has been prepared by a different method which renders it superior in every respect to the one described above. The maximum tolerance for it, as judged by local reactions, has not yet been reached although daily dosages of one half billion organisms are being given. This is fifty times the amount of antigen conveyed by the former vaccine. Small initial doses have been increased rapidly to the present amounts. There have been neither local nor systemic reactions. The antigenic property has not been impaired inasmuch as this man's serum agglutinated *P. tularensis* through a dilution of 1:80 after the first thirty-two injections. For this short injection period this is a considerably higher agglutinin titer than that induced with the other vaccine.

To each 2 cc. of concentrated *P. tularensis* suspension is added 2 cc. of a freshly prepared 30 per cent solution of sodium nitrite and 2 cc. of 30 per cent acetic acid. The mixture is allowed to stand at room temperature over night and is then washed thoroughly with sterile saline solution. The commercially available Mulford concentrate, containing 10 billion organisms per cubic centimeter, is detoxified equally as well as the fresh living bacteria and its use offers the further advantage of approximate standardization of the densities of suspensions that are made from it. This vaccine can be made safely anywhere. Also it permits prophylactic vaccination to become a practicable procedure. I might add that my own serum agglutination titer was 1:80 on October 14, nineteen weeks after the last date of administering this vaccine.

THE EXPERIMENTAL PRODUCTION OF AGRANULOCYTOSIS*

ROY R. KRACKE

From the Department of Bacteriology and Pathology, Emory University School of Medicine, Emory University, Georgia

The disease now known by the terms agranulocytosis, agranulocytic angina, mucositis necroticans agranulocytica, sepsis with granulocytopenia, malignant neutropenia, granulocytopenia, and agranulosis, is probably a distinct clinical entity that has arisen during the past ten years. Occasional cases, however, were observed before 1922, as, for example, the one reported by Brown[2] in 1902, and the infant, reported by Leale in 1910, who in manhood was again observed by Rutledge et al.[2] in 1930.

When Schultz first described this condition as a clinical entity and gave it a name, it was thought by many that it represented only a peculiar and very unusual reaction of the leukocyte picture to some type of infectious disease. It would seem strange, however, that such a reaction would develop within the past decade and have been previously unobserved in any appreciable numbers, especially since blood counts have been done routinely in many large institutions for nearly fifty years. If the condition had existed to any large extent prior to 1922, it would probably have been described long before, since the diagnosis is easily made, as the condition appears suddenly, runs a dramatic course and usually terminates fatally.

Agranulocytosis may be defined as a disease characterized by marked diminution in the number of circulating granulocytes, this in turn followed by no infection, localized infection, or generalized infection. Apparently it is a distinct clinical entity, with a clear cut pathological picture, a definite clinical course,

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, June 7–9, 1931.

with signs, symptoms and a blood picture that is as characteristic as that occurring in pernicious anemia. In agranulocytosis there is a loss of myelocytic activity of the bone marrow with much evidence to indicate that it is primary, followed by a typical blood picture and a definite clinical course.

Much evidence is at hand to indicate that the primary changes are those of the bone marrow, as evidenced by the studies of Roberts and myself,[20] in which we demonstrated the disappearance of the granulocytes for four days prior to the clinical onset. Furthermore, much evidence from necropsy examinations indicates the essential pathological changes to be those of the bone marrow as described by Piette.[18] Bone marrow studies made during the life of the patients have been described by Buck[3] and others. Roberts and I have now studied the bone marrow removed during life from five patients and find an apparent cessation of activity of the myelocytic elements and in some instances the complete absence of those elements although the erythroblastic tissues were normal.

I believe that it can be reasonably assumed that agranulocytosis is primarily a disease of the myeloblastic tissues, followed by loss of resistance resulting in overwhelming infection. If this be true, the next question is, what agent is responsible for the cessation of myeloblastic activity.

There has been much speculation and divergence of opinion as to the cause of this disease. When Lovett[15] first reported the condition in this country in 1922, she found *Pseudomonas aeruginosa* (*Bacillus pyoceaneous*) in the oral lesions, whereupon she attempted to reproduce the granulopenia in laboratory animals with this organism. She succeeded in producing a mild granulopenia, but close study of her results indicates that the degree of granulopenia which she produced could often be observed in normal rabbits and guinea-pigs.

Pseudomonas aeruginosa which I recently isolated from the blood stream of a patient dying with agranulocytosis, failed to disturb the blood picture of rabbits and guinea-pigs. This organism has been frequently observed in ulcers in the mouth and in two instances in the blood stream, and therefore has been sus-

pected as a possible etiological agent, but in no instance, to my knowledge, has it been used successfully to produce agranulocytosis. This does not mean, however, that it should be ruled out as a possible causative factor.

Various other organisms have been suspected as being responsible for this disease, including all of those that have been isolated from the ulcers and blood stream of patients with the disease. In many instances, efforts have been made to produce granulopenia with these organisms, but apparently with little success. I have injected various organisms isolated from the blood stream of patients with the disease, and in each instance have failed to depress the leukocyte count. This, with the failure of others to reproduce the disease with bacteria, is further evidence that some other type of toxic agent depresses the bone marrow, resulting in an overwhelming infection by any or every organism that happens to reside in the gastro-intestinal tract.

There are some who believe that the bone marrow depression is caused by some type of spirillum that normally inhabits the mouth, or by a pathogenic spirillum that may be present in Vincent's angina. This belief has supportive evidence in the large number of patients that give a history of tooth extraction or oral treatment for Vincent's angina prior to the clinical onset. A review of the literature shows that nearly one half of these patients give such a history. This fact, however, can be variously interpreted. It is plausible to assume that these patients have mouth infections because of a lowered cellular resistance of a chronic granulopenia, or because of an unrecognized attack of acute granulopenia. Roberts and I[19] have stressed the occurrence of this condition, which is characterized by weakness, easy fatigue, and tendency to infection, especially in the oral cavity, where infection would most likely occur. It is my opinion that the high incidence of oral infection is a result and not the cause of chronic or acute granulopenia. In my last two necropsy studies of this disease, I examined bone marrow from the humerus, tibia, femur, sternum and ribs by dark field methods and was unable to find any organisms that would not stain with aniline dyes. I have also made dark field studies of five cases on the bone marrow removed during life, with similar results.

Dead bacteria and bacterial toxins also have to be considered as capable of depressing the leukocyte count, as evidenced by Bromberg and Murphy's[1] report of agranulocytosis following prophylactic typhoid inoculations, though it is questionable as to whether or not this was a mere coincidence. I[14] have reported a similar case in which a woman failed to recover from the final dose of typhoid vaccine inoculation, since she remained in bed with daily fever and extreme weakness for thirty days, culminating in three attacks of agranulocytosis.

It may be possible that the bone marrow depression is caused by a hidden or latent infection over a long period of time, so that when the resistance falls to a low ebb, acute infection follows, the myelocytic tissues being unable to meet the emergency. Adding support to this view is the number of cases that follow influenza after a variable period of time. Since the infecting agent of influenza tends to produce a leukopenia, it is quite possible that the residuals of the infection produce a slow and gradual damage to the bone marrow from which it is unable to recover. The low leukocyte count and extreme weakness following attacks of this disease tend to support this view.

In this modern trend of exposure of the human skin to rays of various types, as ultra-violet light, infra-red, sunlight, and roentgen, the possible effect of these substances should be considered. Pappenheim[17] caused leukocytes to disappear from the blood of rabbits by a single injection of thorium-X. Radiologists have long been aware of the leukocyte depressing factors in excessive dosage of roentgen-radiation.

Certain chemicals have long been known to be effective in the production of leukopenia. Arsenical preparations have been reported as being capable of producing this effect. Dodd and Wilkinson[6] have shown that arsenicals will depress the leukocyte count, though not to the point of agranulocytosis. A number of cases of agranulocytosis have been reported as following arsphenamine therapy. Then as a strange paradox, others advise the use of arsphenamine in the treatment of the condition. Talley and Griffith[25] report one of the few cases in negroes as following arsphenamine therapy. Arsenical preparations should be con-

sidered as possible etiological factors until definitely ruled out. Dr. Francis Carter Wood[31] has called my attention to the fact that he has found large amounts of arsenic on apples sold on the streets of New York, this arsenic having its origin in the wide use of insecticide sprays in orchards.

Benzene, however, is the only chemical that has been shown for many years to be capable of producing a marked depression of the leukocyte count to the point of complete agranulocytosis. Therefore it is the outstanding agent that should be considered as causative of agranulocytosis.

The most complete review of benzene poisoning has recently been published by Alice Hamilton.[10] Therefore a general discussion of the substance is unnecessary in this paper.

One of the first observations of the toxic effect of benzene on the myeloblastic tissues is that of Selling,[22] who observed three young girls who had been working in a rubber factory where they had been exposed to benzene fumes and then entered Johns Hopkins Hospital with agranulocytosis complicated by purpura and hemorrhages. Two of these patients died. The bone marrow was hypoplastic. The interesting features of these cases was the early chronicity of the symptoms, followed by sudden loss of neutrophiles and rapid death. This, in spite of the fact that one of them had long before quit her work, illustrating the cumulative and delayed effect of the chemical on the hematopoietic tissues. Selling[23] followed this observation by the injection of benzene subcutaneously in rabbits and succeeded in producing a leukopenia down to 200 cells per cu. mm. He concluded that benzene destroyed white cells of the circulating blood and parenchymal cells of the hematopoietic system, but that the circulating erythrocytes were little affected.

Winternitz and Hirschfelder[30] showed that a pneumonic exudate in benzene treated rabbits contained erythrocytes, fibrin, and endothelial cells but not neutrophiles. Kline and Winternitz[12] demonstrated that the loss of resistance to infection with tubercle bacilli was diminished. Camp and Baumgartner[4] were unable to demonstrate neutrophiles in benzene treated rabbits upon the application of croton oil to the ear, and Hektoen[11]

showed that resistance of benzene treated animals was decreased by loss of neutrophiles, reduction in antibodies and reduction in phagocytic activity of leukocytes present. Simonds and Jones[24] also showed that the immune bodies were much decreased in benzene treated rabbits. The most recent and extensive work on the action of benzene has been that of Weiskotten and his associates[26, 27, 28, 29], in which they showed that benzene produced an aplasia of the myelocytic tissues, a marked granulopenia, and that the life span of the rabbit neutrophile was about four days. They also showed that following the injection of benzene, there was a primary fall of the leukocytes, followed by a rise to normal, then a secondary fall, during which the animal usually died, the two falls being designated as the prophase and the deuterophase. Their injections were made subcutaneously, using 1.0 cc. of equal parts of benzene and olive oil per kilogram of body weight. They demonstrated that the urinary phenols markedly increased during and shortly after benzene administration. It seems, then, that the ability of benzene to produce a fatal outcome, due to a reduction of leukocytes, has been well demonstrated in both man and laboratory animals.

How does acute benzene poisoning differ from agranulocytosis? It has been generally accepted that benzene poisoning is accompanied by toxic effects on all elements of the bone marrow, resulting in decrease of erythrocytes, hypoplastic erythroblastic tissues, marked granulopenia, diminution in platelets, purpura, hemorrhages, and in some instances methemoglobinemia. McCord[16] discussed this differentiation, and Dameshek[5] accepted this view. Duke[7] stated that benzene markedly affects the platelets. However, it is equally true that the chief action of benzene is on the myelocytic tissues, and it is also probable that it can be administered in such dosage to produce marked granulopenia with no apparent effects on either the erythrocytes or the platelets, as shown by experiments described in this paper.

In a consideration of the etiological agents causing agranulocytosis, one must recall that the disease has been observed for ten years, that it occurs chiefly in middle aged women, and in a ratio of four women to one man. There is no age limitation as

it has been observed in infants, and a case reported by Roberts and myself[20] was in a woman of seventy-two years. The disease is not highly infectious and it occurs sporadically. It seems to have no seasonal variation, but since it appears to follow influenza in some cases, it may be more prevalent in the winter. The disease does seem to have geographical limitations, since about 80 per cent of the reports are found in the German and American literature. It is comparatively infrequent in France and England, rare in Italy, and extremely rare in the Orient.

This distribution could be correlated to the usage of organic chemical compounds. The development of chemistry has been rapid within the last ten years, and in Germany and the United States is found the widest usage of organic chemical compounds and aniline dye products.

It has been reported in individuals having various occupations and in many classes of people, school children, housewives, business and professional men and women, laborers, scrubwomen, physicians, nurses, farmers, prostitutes, and ladies and gentlemen of leisure; it seems peculiarly prevalent in the last named class, and it is practically confined to the white race.

The observations that the disease occurs largely in Germany and the United States, that it occurs in middle aged white women, for the most part in women of leisure or women living under good economic conditions, that it is seldom seen in a negro, that most of the patients have a history of previous medical care or treatment with various drugs, that the coal tar series of drugs has its widest range in those parts of the world in which the disease is most frequent, that these drugs and chemicals contain the altered or modified benzene ring, and that benzene is the one outstanding leukocyte depressant, lead me to believe that this substance or its products must be seriously considered as being the cause of this condition.

It is well known among industrial workers that negroes can work with benzene, aniline, dinitrobenzene, et cetera, with less danger of systemic poisoning than white men. Alice Hamilton[9] states, "Women are certainly far more susceptible to benzene poisoning than men."

In the production of leukopenia with benzene, Selling[23] and Weiskotten used equal parts of benzene and olive oil, giving daily injections of 1.0 cc. per kilogram of body weight. The olive oil was used, presumably to delay absorption, and the injections were subcutaneous. This involves a consideration of the mechanism whereby benzene produces its effect on the hematopoietic tissues, and the chief question to be answered was whether or not benzene or one of its oxidation products itself was responsible for the bone marrow damage.

Folin and Denis[8] state that "the oxidation of benzene to phenols and to dioxybenzenes is from a biochemical standpoint very interesting as a definite illustration of the vigorous oxidations which can and do occur in the animal body." They further state that among the various oxidation products the following are the most important: phenol, paracresol, orthocresol, pyrocatechin, hydroquinone, paraoxybenzoic acid and muconic acid.

Their work was based on the constant finding of these so-called urinary phenols during benzene administration. Therefore, it might be assumed that if benzene is injected subcutaneously in the animal tissues, it may undergo oxidation at the site of inoculation, and the oxidation products instead of the benzene be responsible for the toxic effects on the bone marrow. An effort was then made to determine the effect of benzene on the bone marrow by injection of pure benzene into the blood stream.

Experiment I: Three rabbits were given intravenous injections of 1.0 cc., 0.5 cc., and 0.25 cc. of benzene. Each rabbit died immediately.

Experiment II: Three rabbits were given intravenous injections of 0.18 cc., 0.12 cc., and 0.06 cc. of benzene. Each rabbit died immediately after injection.

From these simple experiments it would seem that benzene, when free in the blood stream, is extremely toxic for rabbits, and therefore, it seems unlikely that when the substance is given subcutaneously, it is taken up by the blood stream as such, but more likely it is oxidized to a less toxic product which probably acts upon the myelocytic tissues.

In view of this, it was then decided to determine the effect of the various oxidation products of benzene on the leukocyte count.

EXPERIMENTS

Rabbit 36: Given daily subcutaneous injections of 33 per cent phenol in olive oil for twenty days. It produced no effect on the leukocyte count.

Rabbit 34: Given subcutaneous injections of 1.0 cc., of a 33 per cent solution of tricresol, followed by eight intravenous injections of the same drug. It resulted in no depression of the leukocyte count.

Rabbit 47: Given daily intravenous injections of 0.5 cc. of saturated aqueous solution of orthocresol for five days, followed by the oral administration of 3 grains for four days, producing no effect on the leukocyte count.

Rabbit 48: Given daily intravenous injections of 0.5 cc. of saturated aqueous solution of metacresol for four days, followed by oral administration of 5 grains for one dose. No effect on the leukocyte count.

Rabbit 49: Given daily intravenous doses of 0.5 cc. of saturated aqueous solution of paracresol for eight doses, followed by oral administration of 5 grains for five days. No effect on the leukocyte count.

Rabbit 31: Given a 33 per cent aqueous solution of resorcinol (benzene oxidation product). One cc. subcutaneously for twenty days, five doses intravenously, failed to produce a depression of the leukocyte count.

Rabbit 43: Given daily intravenous doses of 0.5 cc. of a 33 per cent aqueous solution of resorcinol for eight days, followed by the oral administration daily of 5 grains for eight days, producing no effect on the leukocyte count.

Rabbit 32: Given daily subcutaneous injection of a 33 per cent aqueous solution of hydroquinone (0.5 cc.) without effect on the leukocyte count. This was done for twenty-two days, then followed by eight daily intravenous injections, producing no effect on the leukocyte count.

Rabbit 46: Given daily intravenous doses of 0.5 cc. of a saturated aqueous solution of hydroquinone for five days. This was followed by a depression of the leukocyte count to 2,850. The administration of 5 grains daily by mouth for eight days failed to depress the leukocyte count.

Rabbit 33: Given daily subcutaneous injections of a 33 per cent aqueous solution of pyrocatechin for twenty days, followed by the same dose intravenously for eight days. It produced no effect on the leukocyte count.

Rabbit 44: Given daily intravenous injections of 0.5 cc. of 33 per cent aqueous solution of pyrocatechin for six days, followed by seven daily doses of 5 grains by mouth, producing no effect on the leukocyte count.

Rabbit 38: Given daily subcutaneous injections of 1.0 cc. of a saturated solution in 10 per cent alcohol of ortho-oxybenzoic acid (salicycle acid). It seemed to produce a depression of the leukocyte count, at one time reaching 3,300 with 92 per cent lymphocytes. Continuation with intravenous injections failed to affect the count.

Rabbit 41: Given daily intravenous doses of 10 per cent solution of ortho-oxybenzoic acid in 50 per cent alcohol (0.5 cc.) for five doses, this being followed by 5 grains by mouth for eight days. No effect on the leukocyte count.

Rabbit 39: Given daily subcutaneous injections of 10 per cent solution of para oxybenzoic acid in 50 per cent alcohol (1 cc.), followed by intravenous injections. It produced no effect on the leukocyte count, with the exception of mild leukopenia.

Rabbit 45: Given daily intravenous doses of 0.5 cc. of 10 per cent solution in 50 per cent alcohol of para oxybenzoic acid for five days, followed by 5 grains of the drug daily by mouth, producing no effect on the leukocyte count.

Rabbit 42: Given daily intravenous doses of 0.5 cc. of a 10 per cent solution of meta oxybenzoic acid in 50 per cent for six days, followed by 5 grains daily by mouth for fourteen days, producing no effect on the leukocyte count.

Rabbit 40: Given daily subcutaneous injections for twelve days of 1.0 cc. of a 10 per cent solution of meta oxybenzoic acid in 50 per cent alcohol, followed by intravenous injections, for five days. It failed to produce a depression of the leukocyte count.

Rabbit 37: Given daily injections of 1.0 cc. of 50 per cent alcohol subcutaneously, followed by six intravenous doses. No effect on the leukocyte count. This was given as a control, since many of the solutions used were alcoholic.

A summary of the results of the foregoing experiments indicates that intravenous administration of hydroquinone is capable of producing a moderate neutropenia. Also, subcutaneous administration or ortho-oxybenzoic acid, produces moderate neutropenia. Further work remains to be done with these two substances. Intravenous and oral administration of ortho-oxybenzoic acid, and subcutaneous injections of hydroquinone fail to produce this effect. Therefore, in a consideration of the depressing effects of chemicals on the blood forming tissues, the method of administration is very important.

The action of benzene on the myelocytic tissues

The chief object of the following experiments was to observe the action of benzene on the hematopoietic tissues, when given by subcutaneous, intraperitoneal, and skin application methods. Also to determine whether or not agranulocytosis could be produced without affecting the erythrocyte or platelet picture.

Rabbit 2: Female (Table 1). Was given 1 cc. of equal parts of benzene and olive oil subcutaneously daily for nine days. Erythrocyte counts, platelet counts, and studies of the erythrocyte picture, showed no disturbance of these elements. The rabbit died on the fourteenth day with 800 lymphocytes per cu. mm., no granular cells, a normal red cell and platelet count. On the eighth

TABLE 1

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	EOSINOPHILS	BASOPHILS
			Segmented	Juvenile	Banded				
3–18	7,600								
3–19	5,200								
3–20	10,100								
3–23	4,100								
3–24	2,050	4,770,000	2	12	20	4	61		
3–25	1,250	5,020,000				12	85		3
3–26	1,400	5,060,000	1	5		12	75	5	2
3–27	3,850	5,770,000		4		15	61		
3–28	600	4,140,000	4	1	32		63		
3–30	1,150	4,790,000					100		

TABLE 2

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	BASOPHILS
			Segmented	Juvenile	Banded				
3–27	8,000								
3–28	2,750		27		3		70		
3–30	3,150		30		2		68		
3–31	2,800		6				94		
4–1	3,000			5	1	4	88	2	
4–2	4,850								
4–3	5,500		15	10	6	6	60		3
4–4	3,800		32		8		60		
4–6	2,750				2	2	93	2	1
4–7	1,900			4		8	83	5	
4–8	10,250			10		28	59	2	1
4–9	1,300					5	95		
4–10	1,350			6		2	92		
4–11	4,600	4,300,000				24	76		
4–13	5,600	4,450,000	16	32		2	46	4	
4–14	7,200		8	8		20	52		
4–15	19,000	4,600,000							
4–16	13,450	4,950,000							
4–18	2,850	5,120,000							
4–20	1,400	5,000,000				16	84		
4–21	1,850					18	82		
4–22	1,100	4,700,000					100		

day the mucous membranes of the mouth were traumatized, injected and sprayed with an eighteen hour culture of diphtheroid bacilli isolated from the blood stream of a patient with agranulocytosis. At the time of death numerous oral ulcers were present. The condition was identical with agranulocytosis as seen in the human being. At autopsy, blood cultures were positive for a diphtheroid bacillus. Areas of congestion were present along the gastro-intestinal tract. Visceral organs showed cloudy swelling. Bone marrow showed an absence of granular cells. Animal died 3–31–31.

TABLE 3

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	BASOPHILS
			Segmented	Juvenile	Banded				
3–18	5,300								
3–19	10,100								
3–20	6,450								
3–23	7,850								
3–24	1,800	4,400,000	12		12		74		2
3–25	1,250	4,320,000							
3–26	1,350	4,380,000		12		22	61	1	
3–27	4,950	4,400,000					5		
3–28	3,900	4,060,000	32		30		38		
3–30	1,450	4,450,000	4				96		
3–31	1,300	5,080,000					100		
4–1	1,300						100		
4–2	1,150	3,270,000					100		
4–3	3,200					24	76		
4–4	1,850	3,170,000		10		35	55		
4–6	5,150	4,100,000		10		64	26		
4–7	5,100	4,530,000				36	61	3	
4–8	5,000	4,120,000		56		42		2	
4–9	3,600	4,500,000		12		74	14		
4–11	1,800	4,000,000				4	96		

Rabbit 3: Female (Table 2). Given 2.0 cc. of equal parts of benzene and olive oil daily, subcutaneously, for twenty days. Note the three falls in the leukocyte count, the animal dying with a leukocyte count of 1100, 4,700,000 erythrocytes and platelets normal. No hemorrhages were noted either before or after death. Autopsy showed aplasia of the granular cell elements, generalized infection, bilateral pneumonia with pneumococcus from blood stream. The picture was that of clinical agranulocytosis. Animal died on 4–22–31.

Rabbit 4: Female (Table 3). Given daily subcutaneous injections of 1.5 cc. of equal parts of benzene and olive oil daily for twenty days. The picture was

TABLE 4

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	EOSINOPHILS	BASOPHIL	DOSAGE
			Segmented	Juvenile	Banded						cc.
1–22	10,100										0.5
1–24											0.5
1–27	13,500										0.5
1–29	4,450										0.5
1–31	11,500										0.5
2–2											0.5
2–4											0.5
2–6											0.5
2–9											0.5
2–11											0.5
2–13											0.5
2–16											0.5
2–18											0.5
2–20											0.5
2–21	5,550										0.5
2–24	2,900		10	2	5		70	3			0.5
2–25											0.5
2–26	4,450		1		4		76	1		18	—
2–27			9	3	2	3	60	5		8	0.5
2–28	6,200		33	4	4	3	54	7		7	—
3–2			23		2		58	6		11	0.5
3–3	6,200		25	1	12		50		11	1	1.0
3–4			20	1	2	1	39	2	30	5	1.0
3–5	7,500		29	1	1		56	3		10	1.0
3–6			30		8		53	4		5	1.0
3–7	4,500		20	2	4	2	69	3			1.0
3–9	2,850		25		8		58	4	2	3	1.0
3–11	5,650		8				86	6			1.0
3–12	3,200	4,320,000	19		4		76	1			1.0
3–13	4,450		18		6		73			3	1.0
3–14	2,800		24		4		56		12	4	—
3–17	1,700					4	96				—
3–18	2,700					3	97				1.0
3–19	1,250		6				94				1.0
3–20	2,900*		2	2	8		86				1.0
3–23	6,750†		1	33		16	42				1.5
3–25	7,350			33	6		61				1.5

* Two myeloblasts.

† Seven myeloblasts.

TABLE 4—*Concluded*

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	EOSINOPHILS	BASOPHILS	DOSAGE
			Segmented	Juvenile	Banded						cc.
3–26	7,050		15	17	11	2	55				1.5
3–27	12,250		5	39	10	15	31				1.5
3–28	2,300		29	13			51			7	1.5
3–30	2,000			2	14		83	1			1.5
3–31	2,000		4	2	5		89				1.5
4–1	1,400			2	6	2	90				1.5
4–2	800			4	12	2	82				1.5
4–3	1,150	2,800,000				8	92				1.5
4–4	500	2,340,000				5	95				1.5

essentially the same as that of Rabbit 3. Autopsy showed generalized infection with pneumococci, bronchopneumonia, and aplasia of the myeloid system. Animal died on 4–12–31.

Rabbit 5: Female (Table 4). Given subcutaneous injections of 0.5 cc. of equal parts of benzene and olive oil every second day, thus resulting in a chronic granulopenia for thirty-five days. The dose was then increased to 1.0 cc. daily, this reducing the leukocyte count to 1,200 after fifteen days (96 per cent lymphocytes), the dose then increased to 1.5 cc. for twelve days, the animal dying on 4–6–31 with a count of 500 per cu. mm., 100 per cent lymphocytes, 4,340,000 erythrocytes, platelets normal. Autopsy showed aplasia of the myelocytic elements of the marrow, generalized blood stream infection with staphylococci.

Rabbit 6: Female (Table 5). Given daily applications of benzene to the shaved abdomen for thirteen days, resulting in severe inflammatory reaction of that area, and a leukocytosis. The leukocytosis and inflammation persisted for ten days. Beginning at this time the animal was given subcutaneous injections of 0.5 cc. of benzene daily (no olive oil). After twenty-nine days, death occurred on 3–23–31 with 900 lymphocytes per cm., no neutrophiles, 4,430,000 erythrocytes. Autopsy findings similar to Rabbit 5 with pneumococci in blood stream. Apparently the presence of a preëxisting leukocytosis did not prevent the development of agranulocytosis. Olive oil did not seem to be necessary for use with the benzene.

Rabbit 7: Female (Table 6). Given daily intraperitoneal injections of 1.0 cc. of benzene in olive oil for sixteen days. Animal died on seventeenth day (4–10–31) with 1,450 leukocytes, 4,200,000 erythrocytes, platelets normal. Neutrophiles 12, lymphocytes 88 per cent.

Rabbit 8: Female. Given daily inhalations of benzene for ten minutes for

seventy days. It produced no effect on the leukocyte count, though the lymphocytic percentages were rather high. Ten minutes was probably an insufficient inhalation period.

TABLE 5

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	BASOPHILS	DOSAGE
			Segmented	Juvenile	Banded					
1-22	25,050									On skin
1-27	33,200									On skin
1-31	14,650									On skin
2-3	27,300									On skin
2-5	19,800									On skin
2-7	15,400									—
2-10	15,450									—
2-12	21,000									—
2-14	15,000									—
										cc.
2-17	18,100									0.5
2-19	7,950									0.5
2-21	5,900									0.5
2-24	8,100									0.5
2-26	8,250									0.5
2-28	6,600									0.5
3-3	4,450									0.5
3-5	4,450									0.5
3-7	3,300									0.5
3-9	2,250		12		9		77		2	0.5
3-11	3,350		6		6		82	6		0.5
3-12	3,000	4,470,000	16		8		72	1	3	0.5
3-13	2,950		44		18		34		4	0.5
3-14	1,350						100			0.5
3-17	2,500		31	4	18		45	1	1	—
3-18	1,200		3	24	2	6	63	1	1	—
3-19	2,500			15		4	81			0.5
3-20	1,500				1		99			1.0
3-23	900	4,430,000					100			—

Effect of the aniline dyes on the leukocyte count

Because of the wide use of aniline and aniline derivatives in recent years, and because of the possibility that this class of substances may be instrumental in depressing the hematopoietic

tissues, four rabbits were injected with the following substances: aniline, para red, beta-naphthol, paranitraniline.

Rabbit 11: The abdomen was shaved and the shaved area painted daily with aniline for thirteen days, resulting in no disturbance of the leukocyte count. The animal was then given 0.5 cc. of aniline daily by the subcutaneous method for twenty-seven days, which also failed to affect the leukocyte count.

Rabbit 12: The same procedure and dosage, using a saturated alcoholic solution of beta-naphthol, was employed in this rabbit as in Rabbit 11. The leukocyte count was not affected.

TABLE 6

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS
			Segmented	Juvenile	Banded			
3–19	8,000							
3–20	11,500							
3–23	12,000							
3–24	10,600		45	1	12	10	32	
3–26	10,950							
3–27	8,000							
3–28	4,200							
3–30	1,900		38		8		54	
3–31	5,800		45		12	2	34	7
4–1	12,700		53	2	15		30	
4–2	13,150		32		5		61	2
4–3	8,200							
4–4	5,650							
4–6	4,950							
4–7	1,550							
4–8	850			32		4	62	
4–9	2,050	4,340,000	44				55	1
4–10	1,450	4,200,000		12			88	

Rabbit 13: The same procedure and dosage, using a saturated alcoholic solution of paranitraniline, was employed in this experiment, as in Rabbit 11. No effect on the leukocyte count.

Rabbit 14: This animal was treated the same way as Rabbit 11 using a saturated solution of para red dye. No effect on the leukocyte count.

A thorough consideration of the nine cases of agranulocytosis that I have studied reveals the fact that eight of the patients had been taking drugs of the coal tar derivatives prior to the clinical onset. This had occurred so consistently and inasmuch as this

class of drugs contains various modifications of the benzene ring, and in view of their widespread use, it was decided to determine whether or not certain drugs of that class produced any effect on the leukocyte count. The drugs used were phenacatine, amidopyrine, peralga, and dial, four proprietary preparations that had been administered in large quantities to four patients later developing agranulocytosis.

Rabbit 15: Given from 2 to 4 grains of phenacatine daily by oral administration for forty-five days, producing no effect on the leukocyte count.

Rabbit 16: Oral administration of 5 to 10 grains of amidopyrine daily for forty-five days. No depression of the leukocyte count; on the contrary, a persistent leukocytosis was obtained.

Rabbit 17: Given from 2 to 4 grains of peralga daily for forty-five days, producing no effect on the leukocyte count.

Rabbit 18: Given from 2 to 4 grains of dial daily for forty-five days, producing no effect on the leukocyte count.

CONCLUSIONS

1. Agranulocytosis is a clinical entity whose pathology is probably primary in the bone marrow, followed by sepsis which may be either local or general, or followed by no evidence of sepsis.

2. Subcutaneous injections of benzene and olive oil (if given in sufficiently small doses, so as not to affect the erythroblastic tissues) resulted in the development of clinical agranulocytosis in rabbits. The smaller the dose, the more selective became the affinity for the myelocytic tissues. The course of the condition seemed to be similar to that seen in the human, that is, first a neutropenia, then generalized infection from organisms already present, or from organisms introduced.

3. Agranulocytosis with subsequent infection and without infection was produced by the subcutaneous injection of benzene without olive oil and also by the intraperitoneal injection of benzene.

4. Benzene inhalations failed to depress the leukocyte count.

5. The intravenous injection of benzene even in small doses resulted in the immediate death of the animal, so it is probable that oxidation products of benzene are directly responsible for its leukocyte depressing properties.

6. Agranulocytosis can be produced with benzene in a rabbit having a leukocytosis.

7. A marked leukopenia was produced by the subcutaneous injection of ortho-oxybenzoic acid and by the intravenous injection of hydro-quinone.

8. The injection of animals by the subcutaneous and intravenous routes and the oral administration of the following substances failed to depress the leukocyte count: amidopyrine, phenacatine, peralga, dial, resorcinol, pyrocatechin, orthocresol, metacresol, paracresol, phenol, para-oxybenzoic acid, meta-oxybenzoic acid, 50 per cent alcohol.

9. Skin application and subcutaneous injection of the following substances failed to depress the leukocyte count: benzene cleaner, aniline, paranitraniline, para red dye, beta-naphthol.

10. The daily intravenous injection of various organisms isolated from the blood stream of patients with agranulocytosis failed to depress the leukocyte count.

11. The etiology of agranulocytosis is unknown, but the benzene ring must be strongly considered.

12. Those who have the opportunity to study this disease should direct their attention to a careful history of possible contact with granulopenic producing substances, bearing in mind that when the patient consults the physician, he presents the terminal stage of a disease that possibly began months before.

REFERENCES

(1) Bromberg, L., and Murphy, P.: Agranulocytic angina following prophylactic typhoid vaccination. Jour. Am. Med. Assn., **92:** 1266–1267. 1929.

(2) Brown, P. K.: A fatal case of primary acute infectious pharyngitis with extreme leukopenia. Am. Med., **3:** 649–651. 1902.

(3) Buck, R. W.: Agranulocytosis associated with anal ulcer. Jour. Am. Med. Assn., **93:** 1468–1469. 1929.

(4) Camp, W. E., and Baumgartner, E. A.: Inflammatory reactions in rabbits with a severe leukopenia. Jour. Exp. Med., **22:** 174–193. 1915.

(5) Dameshek, W.: Benzene poisoning and agranulocytosis. Jour. Am. Med. Assn., **93:** 712. 1929.

(6) DODD, K., AND WILKINSON, S. J.: Severe granulocytic aplasia of the bone marrow. Report of a case following arsphenamine treatment in congenital syphilis. Jour. Am. Med. Assn., **90:** 663–665. 1928.

(7) DUKE, W. W.: Causes of variation in the platelet count. Experimental results showing the effect of diphtheria toxin, benzol and tuberculin on the platelet count in rabbits. Arch. Int. Med., **11:** 100–120. 1913.

(8) FOLIN, O., AND DENIS, W.: The excretion of free and conjugated phenols and phenol derivatives. Jour. Biol. Chem., **22:** 309–326. 1915.

(9) HAMILTON, A.: Industrial poisons in the United States. New York: MacMillan, 1925, pp. 590.

(10) HAMILTON, A.: Benzene (Benzol) poisoning. Arch. Path., **2:** 434–454, 601–637. 1931.

(11) HEKTOEN, L.: The effect of benzene on the production of antibodies. Jour. Inf. Dis., **19:** 69–84. 1916.

(12) KLINE, B. S., AND WINTERNITZ, M. C.: Studies upon exprimental pneumonia in rabbits. Jour. Exp. Med., **18:** 60–74. 1913.

(14) KRACKE, R. R.: Recurrent agranulocytosis: Report of an unusual case. Am. Jour. Clin. Path., **1:** 385–390. 1931.

(15) LOVETT, B.: Agranulocytic angina. Jour. Am. Med. Assn., **83:** 1498–1500. 1924.

(16) MCCORD, C. P.: The present status of benzene (Benzol) poisoning. Jour. Am. Med. Assn., **93:** 280–283. 1929.

(17) PAPPENHEIM, A., AND PLESCH, J.: Experimentelle und histologieche untersuchungen zur Erforschung der Wirkung des Thorium auf den thierischen Organismus. Ztsch. f. Exper. Path. u. Therap., **12:** 95–108. 1913.

(18) PIETTE, E. C.: Histopathology of agranulocytic angina. Jour. Am. Med. Assn., **84:** 1415–1416. 1925.

(19) ROBERTS, S. R., AND KRACKE, R. R.: Agranulocytosis—its classification. Ann. Int. Med., **5:** 40–51. 1931.

(20) ROBERTS, S. R., AND KRACKE, R. R.: Agranulocytosis—report of a case. Jour. Am. Med. Assn., **95:** 780–786. 1930.

(21) RUTLEDGE, B. H., HANSEN-PRUSS, O. C., AND THAYER, W. S.: Recurrent agranulocytosis. Bull. Johns Hop. Hosp., **46:** 369–389. 1930.

(22) SELLING, L.: A preliminary report of some cases of purpura hemorrhagica due to benzol poisoning. Bull. Johns Hop. Hosp., **21:** 33–37. 1910.

(23) SELLING, L.: Benzol as a leucotoxin. Studies on the degeneration and regeneration of the blood and haematopoietic organs. Johns Hop. Hosp. Reports, **17:** 83–142. 1916.

(24) SIMONDS, J. P., AND JONES, H. M.: The effect of injections of benzol upon the production of antibodies. Jour. Med. Res., **33:** 197–211. 1915.

(25) TALLEY, J. E., AND GRIFFITH, G. C.: A discussion of six cases of agranulocytosis. Med. Clin. North Am., **13:** 1079–1090. 1930.

(26) WEISKOTTEN, H. G.: The normal life span of the neutrophile (amphophile) leucocyte (rabbit). The action of benzol IX. Am. Jour. Path., **6:** 183–190. 1930.

(27) WEISKOTTEN, H. G., AND SCHWARTZ, S. C., AND STEENSLAND, H. S.: The action of benzol. I. On the significance of myeloid metaplasia of the spleen. Jour. Med. Res., **33:** 127–140. 1915.

(28) WEISKOTTEN, H. G., SCHWARTZ, S. C., AND STEENSLAND, H. S.: The action of benzol. II. The deuterphase of the diphasic leukopenia and antigen-antibody reaction. Jour. Med. Res., **35:** 63–69, 1916.

(29) WEISKOTTEN, H. G., AND STEENSLAND, H. S.: The action of benzol. V. The diphasic leukopenia as a polynuclear anophophile phenomenon (rabbit). Jour. Med. Res., **39:** 485–494. 1919.

(30) WINTERNITZ, M. C., AND HIRSCHFELDER, A. D.: Studies upon experimental pneumonia in rabbits. Jour. Exp. Med., **17:** 657–665. 1913.

(31) WOOD, F. C.: Personal communication.

DISCUSSION

T. H. BOUGHTON, Akron, Ohio: While I cannot doubt the experimental production of an agranulocytic syndrome by means of benzene, I greatly doubt the clinical significance of benzene in this condition. We are conducting a large-scale clinical experiment in this field. Due to the concentration of the rubber industry in and around Akron, and the very general employment of benzene in the factories we see a great many cases of benzene poisoning, probably more than at any other place in this country. Yet in spite of constant watchfulness, we have found only two cases of agranulocytosis, neither of them in persons subjected to any form of industrial poisoning.

Furthermore, the blood findings are totally different. Benzene poisoning gives the picture of aplastic anemia: very low red count, a color index of one, a moderate leukopenia, and a normal differential. Only a small proportion of the workers exposed to benzene ever develop benzene poisoning. If benzene could produce agranulocytosis, it seems likely that some of these workers experiencing a sub-toxic exposure would develop the syndrome, but we have not found them, and all persons in our factories exposed to benzene are subjected to frequent blood examination.

A SIMPLE MEDIUM FOR THE ISOLATION OF BACTERIA OF THE TYPHOID AND DYSENTERY GROUPS

JAMES C. SMALL AND WILLIAM A. KREIDLER

Laboratory of Bacteriology, Philadelphia General Hospital, Philadelphia, Pennsylvania

In laboratory cultures at the Philadelphia General Hospital for the past six years, the following medium for the isolation of bacteria of the typhoid and dysentery groups has been found to be of advantage over the Endo agar medium now generally used. The medium consists of the following ingredients:

Beef extract	3 grams
Peptone	10 grams
Sodium chloride	5 grams
Agar	25 grams
Lactose	10 grams
Water	1000 cc.

Heat until constituents are dissolved and filter through cotton and gauze. Place in flasks or tubes of convenient size and sterilize in the autoclave at fifteen pounds pressure for fifteen minutes.

Indicator: Dissolve 0.25 gram of brom-thymol-blue in 100 cc. of 95 per cent ethyl alcohol. Add 2 cc. of the indicator to 100 cc. of medium just before plates are poured.

The plated medium is light green in color and remains suitable for use over a period of weeks if stored in the ice-box. A loopful of emulsified feces or of the sediment of centrifugalized urine is streaked over the surface of the medium. After eighteen to twenty-four hours incubation at 37°C., the lactose-fermenting microörganisms produce yellow colonies and non-lactose-fermenters appear as blue colonies. A zone of the medium adjacent to the colonies likewise assumes these colors respectively.

The medium keeps indefinitely and is not affected by light. The tinted colonies are shaply defined and differentiated. Each

retains its particular color, even though they may occur adjacently on the plates.

Brom-thymol-blue exhibits no inhibitory action on bacteria of the colon-typhoid-dysentery groups, therefore these microorganisms grow as readily on this medium as they do on plain lactose-agar. This feature makes the medium especially serviceable for the primary isolation of bacteria belonging to the dysentery group.

A PRECIPITIN TEST FOR THE DIAGNOSIS OF MENINGITIS

WILLIAM A. KREIDLER AND MARGARET E. MURPHY

From the Bacteriological Laboratory of the Philadelphia General Hospital, Philadelphia, Pennsylvania

It is obviously of importance in the treatment of infections of the meninges that the laboratory diagnosis be made in the shortest possible time and that the commercial serum possessing the greatest specificity for the particular strain of microörganism causing the infection be selected for treatment.

For the past year, in the routine testing of spinal fluid from patients suffering from meningitis, we have employed a precipitin test on the clear spinal fluid after the bacteria and other cells have been removed by centrifugalization. We have proceeded on the assumption that in the spinal fluid of meningitic patients there is present a soluble precipitable substance (precipitinogen), derived from the invading microörganism, which is thrown out of solution when the spinal fluid is mixed with an immune serum containing precipitins for the particular strain causing the infection.

TECHNIQUE OF TEST

Spinal fluid from patients with suspected meningitis is centrifugalized at high speed for twenty minutes. The clear supernatant liquid is removed to another test tube by means of a capillary pipette. Smears and cultures may then be prepared from the sediment in the usual manner. From 0.3 to 0.5 cc. of each available commercial or stock serum is placed in small test tubes. An equal volume of supernatant fluid, which has been separated from the spinal fluid sediment, is layered over the serum by means of a capillary pipette. In less than twenty minutes, the precipitinogen in the spinal fluid will unite with any homologous precipitins in the serum to form a delicate but distinct white ring at the line of contact (Figs 1 and 2). After standing over night at room temperature, a precipitate is thrown to the bottom of the tube (or tubes) which showed an immediate ring test. The amount of precipitation may be graded

from + to ++++. Control tests using normal spinal fluids failed to give either the white ring or the precipitate.

The test is not only a rapid and satisfactory method of determining the etiology of the meningitis (meningococcic, pneumo-

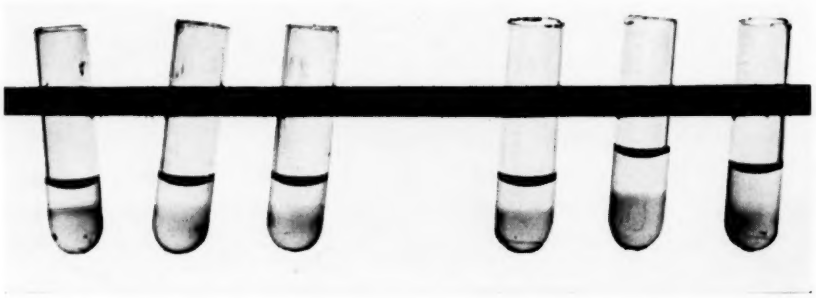

FIG. 1. SPINAL FLUID PRECIPITIN TEST WITH COMMERCIAL ANTIMENINGOCOCCIC SERUMS (X, Y, Z) IN A CASE OF MENINGOCOCCIC MENINGITIS

Appearance after standing twenty minutes. A precipitate forms which sinks to the bottom of the tube after eighteen hours.

FIG. 2. SPINAL FLUID PRECIPITIN TEST WITH ANTIPNEUMOCOCCIC SERUMS IN A CASE OF PNEUMOCOCCIC MENINGITIS

Appearance after standing twenty minutes. A precipitate forms which sinks to the bottom of the tube after eighteen hours.

coccic, et cetera) but the density of the ring and the amount of the precipitate indicate which of the commercial serums available

TABLE 1

RESULTS OF PRECIPITIN AND AGGLUTININ TESTS ON 16 SPINAL FLUIDS FROM CASES OF MENINGOCOCCIC MENINGITIS

NO.	PRECIPITIN TESTS USING UNDILUTED COMMERCIAL ANTIMENINGOCOCCIC SERA			AGGLUTININ TESTS USING UNDILUTED COMMERCIAL ANTIMENINGOCOCCIC SERA		
	X	Y	Z	X	Y	Z
1	++++	+	+	1:80	−	−
2	++++	++	++	1:80	−	−
3	++++	−	−	1:40	−	−
4	++++	−	−	1:160	−	−
5	++	−	−	1:160	−	−
6	++++	++	−	1:640	1:80	−
7	++++	+	+	1:160	1:40	1:20
8	++++	++	+	1:320	−	−
9	++++	−	−	1:160	−	−
10	++++	−	−	1:80	−	−
11	++++	++	−	1:320	1:20	−
12	+++	−	−	1:80	1:20	−
13	++++	−	−	1:80	−	−
14	++++	−	+	1:640	1:80	1:80
15	++++	+	−	1:320	1:80	−
16	+++	−	−	1:320	−	−

Very strongly positive, ++++; strongly positive, +++; positive, ++; weakly positive, +; no precipitation or agglutination, (−). The figures represent the highest dilutions of commercial serums which agglutinated the isolated meningococci (in bouillon). Commercial Sera, −X, Y and Z. All controls were negative.

TABLE 2

RESULTS OF BACTERIOLOGIC AND SEROLOGIC TESTS ON 31 SPINAL FLUIDS

NO. OF SPINAL FLUID	RESULT OF SMEAR	RESULT OF CULTURE	PRECIPITIN TESTS			AGGLUTININ TESTS		
			Anti-meningococcic serum	Antipneumococcic serum	Anti-streptococcic serum	Anti-meningococcic serum	Antipneumococcic serum	Anti-streptococcic serum
15	Meningococcus	Meningococcus	Pos.	Neg.	Neg.	Pos.	Neg.	Neg.
5	Meningococcus	No growth	Pos.	Neg.	Neg.	No agglutinin tests possible		
1	Pneumococcus	Pneumococcus	Neg.	Pos. Type III	Neg.	Neg.	Pos. Type III	Neg.
1	Streptococcus	Streptococcus	Neg.	Neg.	Neg.*	Neg.	Neg.	Neg.*
9 (normal)	Negative	Negative	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

* No homologous serum available.

possesses the highest precipitin titre for the particular strain of microörganism causing the infection (table 1). Agglutination tests performed with the same serums and broth cultures of the isolated organisms have verified the results of the precipitin tests in each case tried (table 2). In five cases studied, in which the precipitin tests were positive, the agglutination tests could not be performed because the cultures were negative. In such cases the precipitin test possesses a distinct advantage.

SUMMARY AND CONCLUSIONS

A rapid and accurate precipitin test for use in the diagnosis of meningitis is described.

The precipitin test on meningitic spinal fluids renders possible the reporting of the causative organism and also the commercial serum most suitable for treatment within thirty minutes after the specimen reaches the laboratory.

The precipitin tests have been checked with agglutination tests whenever possible and results have agreed consistently.

In five cases studied in which the cultures were negative, thus rendering an agglutination test impossible, the precipitin test was positive. This fact and the possibility of returning an almost immediate report are advantages of the precipitin test in the diagnosis of meningitis that justify its use in the routine examination of spinal fluid in cases of suspected meningitis.

AN ANALYSIS OF 1535 AUTOPSIES*

OSBORNE ALLEN BRINES

From the Department of Pathology, Receiving Hospital, Detroit, Michigan

The following report is based on 1535 consecutive autopsies performed at Receiving Hospital during the past four years. The prevalence of various fatal diseases in this summary is doubtless influenced by prevailing hospital conditions. Receiving Hospital is a general hospital with a daily census fluctuating between 650 and 875 patients, the variation being due principally to conditions on the psychopathic service where there are between 200 and 400 patients. All other services are represented except obstetrical and contagious; the pediatric department is small. The main services, in addition to the psychiatric, are general surgery, general medicine and gynecology. It must be expected that the age of patients coming to autopsy in this series is higher than in most general hospitals.

Although Receiving Hospital maintains the principal emergency station in Detroit very few accident cases come to autopsy because most of such cases come under the jurisdiction of the Coroner and are immediately removed to the County Morgue either because of the medicolegal aspects presented or because the period of hospitalization was less than thirty-six hours.

I realize that the same autopsy material might easily yield different results in the hands of some other reviewer and it is not unlikely that different conclusions might be reached by the same individual a few years later due to changing view points and interpretations. It is also easily possible that a similar survey of autopsy material in another general hospital in the same community would present a totally different distribution of types of disease because of different clinical or staff conditions.

*Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

Seventy-six per cent of these autopsies were performed on males and there were twice as many white males as colored. Sixty per cent of the females were white. The average age of all patients was 42.7 years; that of white males 46.2, white females 41.1, colored males 39, and colored females 35.7. Eleven infants

TABLE 1

MAJOR LESIONS ENCOUNTERED AT AUTOPSY*

	NUMBER	PER CENT
Cardiac disease	315	20.0
Tuberculosis	223	15.0
Pneumonia	216	14.0
Renal disease	208	14.0
Syphilis	129	8.4
Malignancy	127	8.0
Peritonitis	94	6.0
Intracranial hemorrhage	90	5.9
Meningitis	64	4.2
Hepatic disease	48	3.1
Brain infarction	30	2.0
Septicemia	27	1.8
Intestinal obstruction	26	1.8
Diabetes	19	1.2
Anemia	12	
Intracranial abscess	10	
Ulcerative colitis	9	
Hemorrhage	7	
Thyrotoxicosis	6	
Splenic disease	5	
Addison's disease	4	
Cause undetermined	109	7.0

* In some instances more than one major lesion was found at autopsy or two classifications were possible for example, heart disease and syphilis, cardiac and renal disease, tuberculosis and peritonitis, et cetera.

whose age was less than one year and two fetuses were not counted in arriving at this average. Considering the high percentage of middle aged and elderly males, the unusually high incidence of some diseases and the low incidence of others is not surprising.

Of the 1535 cases, the cranial cavity was examined in 1070 cases, or 70 per cent. When a well established clinical diagnosis ruled

out the probability of intracranial lesions the examination of the head was omitted. It is not likely that many lesions were missed as the result of this practice. Table 1 illustrates the distribution of major fatal lesions. Cardiac lesions head the list as the greatest cause of death in this series. Table 2 gives the total number and the different types of cardiac cases encountered. I realize that the term "hypertensive heart" might elicit critisism from pathologists for being too clinical, but I have attempted to correlate clinical and pathological findings in arriving at a final diagnosis in order to make results more valuable to the clinical staff. In the hypertensive group were placed those cases of

TABLE 2

CARDIAC DISEASE

315 cases or 20 per cent of all autopsies

	NUMBER	PER CENT
Hypertensive heart	131	40
Coronary sclerosis	46	15
Bacterial endocarditis	39	13
Rheumatic heart	34	11
Pericarditis	27	9
Syphilis	22	6
Coronary occlusion	16	5
Myocarditis (active)	8	
Hemopericardium	2	

marked left ventricular hypertrophy with no valvular lesion and with coronary sclerosis of no, or only secondary, importance. In 15 per cent, or forty-six of the heart cases, coronary sclerosis without demonstrable occlusion was the most important lesion, and the underlying factor in the death of the patient. Perhaps these two groups should have been combined because it is often impossible from the autopsy material alone to classify accurately and separate hypertensive heart disease and coronary sclerosis.

In twelve of the twenty-seven cases of pericarditis there was suppuration and nearly all of these were due to pneumococcic infection which was the result of pleural extension. There were eight cases of adhesive pericarditis which were non-rheumatic,

nearly all of which were associated with cardiac hypertrophy, secondary myocardial degeneration and final cardiac failure. The remaining seven of the twenty-seven cases were cases of tuberculous pericarditis.

Six of the cases of myocarditis were presumably bacterial while the remaining two were suspected of being syphilitic, although spirochetes were not demonstrated. Of the sixteen instances of coronary occlusion, twelve were old, two having resulted in left ventricular aneurism; the remaining four were cases of recent coronary thrombosis. This is a surprisingly low number of cases of coronary occlusion but because of the suddenness of death this type of case is quite likely not to come to autopsy at the hospital. In the sixteen cases of coronary occlusion there was no reason for suspecting syphilitic infection in thirteen, whereas in the remaining three there was either clinical, histological or serological proof of syphilis.

The high incidence of advanced renal arteriosclerosis associated with hypertensive heart disease is instructive (table 3); as far as could be ascertained, sclerosis of the large renal vessels occurred about as frequently as sclerosis of the arterioles. The average age of patients coming to necropsy with hypertensive heart disease was fifty years and 90 per cent occurred in males. No other type of cardiac disease seemed to be greatly influenced by the sex of the patient. In two cases of marked diffuse cardiac hypertrophy, the cause could not be discovered.

In the thirty-four cases of rheumatic heart infection, the inflammatory reaction was in a chronic stage in thirty. Table 3 also gives the complications of rheumatic carditis. The average age of the patients dying of rheumatic heart infection was thirty-eight years and 73 per cent were males; this was the percentage of males in all autopsies. Adhesive pericarditis complicated rheumatic carditis eleven times or 30 per cent which is a much lower figure than was anticipated.

There were two cases of vegetative tricuspid valvulitis, one of which was due to gonorrheal infection. The average age of the patients dying from bacterial endocarditis was forty-three years and 75 per cent were males.

Renal arteriosclerosis was present in only five cases, or 9 per cent of coronary sclerosis (table 3) as contrasted to 82 per cent in hypertensive heart disease.

The position is sometimes taken that many cases of aortic stenosis which is usually attributed to arteriosclerosis are really

TABLE 3

CARDIAC DISEASE COMPLICATIONS

	NUMBER	PER CENT
Hypertensive heart (131 cases)		
Renal arteriosclerosis	108	82
Coronary sclerosis	12	10
Syphilitic aortitis	3	
Unclassified	8	
Coronary sclerosis (46 cases)		
Cardiac hypertrophy	23	50
Syphilitic aortitis	4	8
Renal arteriosclerosis	5	9
Aortic stenosis	8	17
Unclassified	6	
Rheumatic heart (34 cases)		
Mitral stenosis	14	41
Aortic and mitral stenosis	9	27
Aortic stenosis	3	9
Unclassified	8	
Bacterial endocarditis (39 cases)		
Mitral valve	20	51
Aortic valve	11	30
Aortic and mitral	6	16
Tricuspid	2	

the end results of rheumatic infection. Considering the age, however, of these eight patients together with the absence of inflammatory changes, the marked coronary sclerosis present and the low incidence of aortic stenosis found in typical rheumatic heart disease, it would seem rather poor logic to consider aortic

stenosis, which is found associated with coronary sclerosis, in individuals of middle age or past, as being on a rheumatic basis. The average age for this group was fifty-eight years with 75 per cent males.

The wide range in age incidence in all types of heart disease contributes little to facilitate clinical diagnoses. In the hypertensive group there were five patients aged between twenty and thirty years, three of these cases being associated with glomerulonephritis; there were twenty patients between thirty and forty years, while ten patients were aged seventy or more years. Fourteen of the thirty-three cases of rheumatic heart disease occurred

TABLE 4

TUBERCULOSIS*

223 cases or 15 per cent

Pulmonary		169 cases
Bilateral	146	
Left only	13	
Right only	10	
Generalized miliary		52 cases
Tuberculous meningitis		11 cases
Tuberculous peritonitis		10 cases
Tuberculous pancreatitis		5 cases
Tuberculous myocarditis		3 cases

* Some cases had more than one tuberculous lesion; for instance, one case may be listed under miliary, pulmonary and peritonitis. The greater number of cases than lesions is thus accounted for.

in patients between the ages of forty and fifty years whereas three patients were older. One case of advanced coronary sclerosis was found in a colored male aged twenty-five years; five other cases were in patients in the next decade. In two cases of coronary thrombosis the patients were aged thirty-three and thirty-five years.

A patent foramen ovale was found in two infants and in a white male aged forty-six years. A patent ductus arteriosus was found in an eleven months old child.

Tuberculosis accounted for 223 cases, or 15 per cent, of all deaths, and ranked second as a fatal disease. Table 4 may be

consulted for the distribution of lesions. In fifty-eight cases, or 34 per cent, of the pulmonary tuberculosis there was severe terminal tuberculous infection of the liver while infection of the myocardium was found only three times and of the pancreas five times.

Pneumonia accounted for the third largest number of deaths. In this group there were 216 cases, or 14 per cent, of all deaths. One hundred eighty-eight of these were lobar and twenty-five bronchopneumonia. Of the 171 cases of lobar pheumonia in which the side involved was recorded, there were thirty-seven, or 22 per cent, in which the lesion was bilateral, thirty-nine, or 23 per cent, with left side involvement only and ninety-five, or 55 per cent, in which only the right lung was involved. In ten additional cases suppurative pleurisy accounted for death.

Renal disease ranked fourth as a fatal lesion. Table 5 shows the incidence of the various types of renal lesions. Eighteen of the twenty cases of glomerulonephritis could readily be classified as chronic. In three instances hypertensive heart disease was associated with and apparently the result of glomerulonephritis. In four, or 20 per cent, of the cases of glomerulonephritis there was rheumatic heart disease and a common etiology in these instances cannot but be considered. In five additional cases of glomerulonephritis there was superimposed renal arteriosclerosis. One patient with chronic glomerulonephritis died of eclampsia.

Fourteen, or 7.4 per cent, of the cases of non-suppurative renal disease presented a clear cut chronic or subacute inflammatory lesion apparently the result of bacterial infection. The microscopical picture was an infiltration of inflammatory cells which were chiefly lymphocytes with a few polymorphonuclears, plasma cells and eosinophiles, associated with some connective tissue hyperplasia. In half of these the inflammatory picture was diffusely distributed whereas in the other half there was focal distribution. These I designated as stromal, or interstitial nephritis; those with the diffuse distribution roughly resembled the nephritis following scarlet fever although the history in none of the cases revealed previous acute infection. It is further interesting to note that in four of these twelve cases jaundice was an outstand-

ing symptom and that a definite active chronic hepatitis was usually present.

The complications of the 131 cases of vascular nephritis were distributed as follows: sixty-eight, or 52 per cent, were associated with hypertensive heart disease and fifteen, or 11 per cent, with coronary sclerosis. In other words, about half of the cases of renal arteriosclerosis occurred without hypertensive heart disease whereas the latter was nearly always associated with severe vascular disease of the kidney. In eight, or 6 per cent, of the cases of renal arteriosclerosis there was intracranial hemorrhage and in nine additional cases there was both hypertensive heart disease and cerebral hemorrhage. No cases of classical nephrosis were encountered in this series. Congenital polycystic kidneys

TABLE 5

DISTRIBUTION OF RENAL DISEASE

208 cases or 13 per cent

	NUMBER	PER CENT
Renal arteriosclerosis	131	63.0
Glomerulonephritis	20	10.0
Suppurative	19	9.0
Stromal nephritis	14	7.0
Glomerular and arteriosclerosis	5	2.4

were found in two cases and horseshoe kidney was found twice. There were six additional cases of severe pyogenic infection of the lower urinary tract resulting in the death of the patient.

During the past year solitary cyst of the kidney was an accidental autopsy finding in seven cases. These cysts ranged in size from 5 to 18 cm. in diameter. This suggests that if more pathologists would report solitary cysts of the kidney encountered routinely, the lesion would lose, to a large extent, some of the reputation which it now enjoys of being a rare lesion.

Syphilis assumed fifth place with 129 fatal cases, or 8.4 per cent, of all deaths. Table 6 shows the type of fatal syphilitic lesion present. Eighty-two, or 38 per cent, of all cases of syphilis, or 64 per cent of all fatal syphilis, showed evidence of central nervous

system involvement and of these eighty-two cases, fifty-nine, or 79 per cent, were diagnosed syphilitic meningo-encephalitis, the clinical diagnosis of which was usually paresis. Gumma of the brain was found twice and of the liver three times. Of the eighty-two cases of cerebral syphilis there was no evidence of syphilitic aortitis in twenty-seven, or 32 per cent. The large number of cases of neurosyphilis is, of course, due to the large number of psychopathic patients housed in this hospital and would not be encountered in the autopsy work of the average general hospital.

TABLE 6

SYPHILITIC INFECTION

216 cases

Neurosyphilis (Meningoencephalitis—59)	82 (38 per cent)
Neurosyphilis without aortic lesion	27 cases
Thoracic aneurism	19 (9 per cent)
Syphilitic aortitis	12 per cent of all autopsies

FATAL CASES OF SYPHILIS

129 or 8.4 per cent of all autopsies

Neurosyphilis	82 cases
Aortic valve lesion	22 cases
Cerebral hemorrhage (syphilitic)	8 cases
Cerebral infarction (syphilitic)	8 cases
Ruptured aneurism	6 cases
Hereditary syphilis	5 cases

Deducting the eighty-two cases of death from neurosyphilis which would bring conditions roughly down to the level encountered in most general hospitals, syphilis would then be reduced from fifth to eleventh place as a cause of death or 3 per cent of all autopsies instead of 8.4 per cent.

In 216, or 15 per cent, of all autopsies there was histopathological evidence of syphilis. Syphilitic aortitis was found in 184 cases, or 12 per cent, of all autopsies. Aortic aneurism on a syphilitic basis was found nineteen times, or in 10 per cent of the cases of syphilitic aortitis; only six of the aneurisms had ruptured.

There was syphilitic heart disease (aortic valve involvement) in twenty-two cases, or 12 per cent, of 184 cases of syphilitic aortitis. In three additional instances there was combined aortic aneurism and aortic regurgitation on a syphilitic basis. Syphilitic aortitic infection (ruptured aneurism or aortic regurgitation or both) caused death in thirty-one instances, or 17 per cent, of the cases of syphilitic aortitis which was only 2 per cent of all the cases autopsied. Routine Wassermann and Kahn tests in this hospital on patients admitted averaged 13.7 per cent positive.

A study of a group of 105 cases of syphilitic aortitis showed that the diagnosis was made at the autopsy table in only fifty-

TABLE 7

MALIGNANT DISEASE

127 cases (8 per cent)

Carcinoma	93 (74 per cent)
Sarcoma	9 cases
Melanoma	4 cases
Hodgkin's disease	4 cases
Glioma	4 cases
Leukemia	4 cases
Meningioma	4 cases
Teratoma of testis	2 cases
Pituitary tumor	2 cases
Auditory nerve tumor	1 case

eight instances, or 53 per cent. The difficulty in making a diagnosis of syphilitic aortitis from gross specimens was apparently due, at least to some extent, to the high age average of the postmortem subjects and therefore to the presence of superimposed aortic atherosclerosis. In the nineteen cases of aortic aneurism, cardiac hypertrophy occurred in six, or 32 per cent. Of the 184 cases of syphilitic aortitis, active tuberculous infection was found twenty times, or 11 per cent, which was 4 per cent less than the incidence of tuberculosis in all cases autopsied. It should be stated that nearly all of the syphilitic patients in this hospital are untreated. There were five cases of hereditary syphilis, the ages being twelve days, three months, six months, two years and three years.

Malignant disease ranked sixth as a cause of death with 127 cases, or 8 per cent. Table 7 presents the frequency with which the various types of malignancy were encountered.

Carcinoma of the stomach occurred twenty-five times with metastasis to the liver in thirteen of these cases. Twenty-two of these twenty-five cases occurred in white males and two in colored males. Carcinoma of the stomach appeared to follow an old peptic ulcer in two cases, or 8 per cent. Primary carcinoma of the lung and of the large intestine came next in order of frequency, there being eleven of each. Of the eleven carcinomas

TABLE 8

CARCINOMA OR 93 CASES

Stomach	25 cases
Lung	11 cases
Intestine	11 cases
Uterus { cervix 2, body 7 }	9 cases
Prostate	8 cases
Esophagus	6 cases
Liver	5 cases
Pancreas	5 cases
Kidney	4 cases
Thyroid	3 cases
Breast	2 cases
Bladder	2 cases
Brain	1 case
Gall bladder	1 case

of the lung, six were the small spindle, oat cell or columnar cell type, while three were squamous and two adenocarcinoma. Of the eleven cases of carcinoma of the intestine, seven occurred in the rectum and two of these were squamous. In Table 8 there are additional figures relative to the location of cancerous lesions. Of the three cases of carcinoma of the bladder, skeletal metastasis was found in two. Primary carcinoma of the liver was found five times, two of which were cholangiomas and three hepatomas. There was one carcinoma of the gall bladder.

In six carcinomas of the esophagus, two showed no evidence of metastasis. There were four cases of carcinoma of the kidney,

two of which were interesting in that there was bladder implantation. In one case multiple malignant adenomas were found in both kidneys. Another papillary carcinoma of the kidney had metastasized to the regional lymph nodes and to the opposite adrenal. There were three cases of carcinoma of the thyroid gland, one of which contained large areas of spindle cells. There were five cases of carcinoma of the pancreas, three occurring in the head and two in the tail. There were two patients with carcinoma of the breast, both of whom had had breast amputations. There was one carcinoma of the cerebellopontine angle, the source of which was undetermined. There was only one instance of a sarcoma growing in a uterine myoma which is remarkable in that during this period 443 specimens of uterine myoma were examined in the laboratory, but only seven of these showed a suspicion of early malignancy microscopically.

Acute generalized peritonitis was encountered ninety-four times, or 6 per cent of all autopsies, and of the ninety-four cases thirty-seven, or 40 per cent, followed operation. No cases of acute gangrenous or ruptured appendix or ruptured peptic ulcer were included in this 40 per cent whether operation had been performed or not. Of the thirty-nine cases of postoperative peritonitis seventeen, or 44 per cent, followed pelvic surgery. In most of these the inflammatory activity of the tissue removed exceeded the postoperative anticipation of the surgeon. It might be added that the greatest conservatism is practiced on the gynecological service of this hospital and that in spite of this conservatism, pelvic surgery heads the list as a cause of fatal postoperative peritonitis. This is a point which many surgeons apparently do not fully appreciate. Twenty-six per cent, or ten cases, of postoperative peritonitis followed the removal of non-suppurative appendices while six cases followed gastro-intestinal operations and four followed gall bladder surgery. Hernial repair and abdominal paracentesis each contributed one case of postoperative peritonitis. Of the remaining fifty-five cases of generalized peritonitis which did not follow operation, or in which operation was performed only after rupture of a viscus had occurred, twenty-one, or 38 per cent, resulted from an acute

infection or gangrene of the appendix with or without perforation, while eleven or 22 per cent, resulted from perforation of a peptic ulcer which were about equally divided between gastric and duodenal. One case of gastric ulcer occurred in a syphilitic patient and there was impressive histopathological evidence of syphilis in the ulcer base but spirochetes could not be demonstrated.

Intracranial hemorrhage was a fatal lesion ninety times, or 5.9 per cent, of all autopsies. Only four of these were associated with skull fracture and only one was produced by bullet wound. It has been previously pointed out that the low percentage of traumatic cases was due to the fact that the majority of such cases are taken care of by the coroner. In ninety cases of brain hemorrhage thirty-eight, or 42 per cent, appeared to be definitely on an arteriosclerotic basis and in three of these ages were thirty-three, thirty-five and twenty-nine years. Six or 7 per cent of the brain hemorrhage cases had hypertensive heart disease. Cerebral syphilis, however, was present in the surprisingly low number of eight, or 9 per cent. There were eight cases of brain hemorrhage, the cause of which could not be definitely determined. In three of these encephalitic lethargica was probably present. In others there was a question of syphilis while in one case there was an history of alcoholism. These cases of intracranial hemorrhage will be made a subject of a special study to be reported later. A rough survey, however, seems to indicate that an age of fifty years is not a valuable clinical dividing line between brain hemorrhage on an arteriosclerotic basis and that on a syphilitic basis.

There were sixty-four cases of meningitis, or 4.2 per cent, of all cases. The etiology was undetermined in twenty, seventeen were meningococcic, thirteen pneumococcic and five staphylococcic. Mastoid or middle ear infection accounted for nine of the fifty-five cases of suppurative meningitis. In two instances purulent meningitis was superimposed on syphilitic meningitis. Besides the fifty-five cases of suppurative meningitis, there were three of tuberculous meningeal infection and three of chronic leptomeningitis, the etiology of which could not be determined.

Pathologic lesions of the liver probably deserve a more prominent place as causes of death than statistics would indicate; how-

ever, death was attributed to liver disease in forty-eight cases, or 3.1 per cent. There were twenty-six cases of cirrhosis of the liver, twenty-four of which were portal, one biliary and one toxic. This hospital has a large number of patients which are classified as chronic alcoholics and the low incidence of alcoholic cirrhosis of only six cases a year appears surprisingly small. There were twelve instances of liver abscess, nine of which were multiple and three of these were secondary to appendiceal infection. Of the three solitary abscesses of the liver, two were amebic. Four cases of acute yellow atrophy of the liver occurred. There were six interesting cases of severe subacute diffuse hepatitis, three of which were associated with severe gall bladder infection. In two of these there was conspicuous atrophy and necrosis which would classify this lesion very close to subacute yellow atrophy.

Toxic degeneration of the liver, as a secondary or terminal condition, was noted in 104 cases. Only the severe cases, with parenchymatous damage and extensive intracellular infiltration of fat which had progressed to the point where it seemed reasonable to believe that the function of the liver was seriously impaired were included. Further classifying these cases of liver degeneration I found that the largest number (twenty-one, or 20 per cent) were associated with "alcoholic" poisoning. The toxic agent in these cases, however, was probably not the ethyl alcohol but accidental poisons peculiar to some beverages. Sixteen additional cases of severe liver damage were associated with lobar pneumonia, fifteen with tuberculosis, ten with acute peritonitis and seven with brain hemorrhage. In six cases there was no apparent cause for the liver damage while three additional cases were infants where the pathology was not clear cut. Chemical poisoning was responsible for four more, two of which were from morphine and one from lysol. There can be little doubt that the liver damage resulting from bacterial and chemical toxins is a big mortality factor.

Cholelithiasis was an incidental finding, apparently not producing symptoms, in thirty-six cases, or 2.4 per cent. There were three cases of hepatic duct calculi. These soft, easily fragmented masses between 2 and 3 cm. in diameter were found in both the

hepatic and common duct. In spite of these impacted calculi in the common duct there was no appreciable jaundice in any of these three cases.

Definite lesions of the spleen were rather rare. There was one case of Banti's disease, two of traumatic rupture, two of amyloid disease, while in one case extensive tuberculosis was found with the spleen the only organ involved.

There were thirty cases of brain softening without hemorrhage, constituting 2 per cent of all autopsies. Fifteen of these were on an arteriosclerotic basis, eight the result of syphilitic endarteritis, four associated with aneurism of the intracranial vessels and in three the cause was not determined.

General septicemia was a definite diagnosis in twenty-seven cases, eight of which were staphylococcic.

In twenty-six cases of intestinal obstruction which had resulted in the death of the patient, fourteen were postoperative and four of these followed appendectomy, four followed hernia repair and two after pelvic operations. Of the twelve cases which did not follow operation, five were due to strangulated hernia and three to old pelvic infection.

Nineteen cases of diabetes mellitus occurred in eleven females and in eight males with an average age incidence of forty-six years. The earliest mortality from diatetes occurred at the age of fifteen years and the oldest patient was seventy. Microscopic changes in the pancreas could be detected in only six, or 35 per cent. Gangrene of the extremities was found in three of the nineteen cases.

Primary anemia was the cause of death in twelve cases and six of these were typical cases of uncomplicated pernicious anemia. One case was diagnosed pernicious anemia but there was a bacterial endocarditis and in another case the anemia was associated with syphilis. In two other cases a diagnosis of atypical pernicious anemia was made and one of these patients was a twenty-three year old white male. In two other cases the cause of the anemia was undetermined. A diagnosis of pernicious anemia, therefore, could really be made in only six of these twelve cases.

Brain abscess was found ten times and five of these were asso-

ciated with mastoid infection while in three others there was other sinus infection. The two remaining cases followed hernia operations.

Hemorrhage as a cause of death was found seven times, three of which were postoperative. One was traumatic abdominal hemorrhage and another was due to omental and mesenteric thrombosis. There was one case of hemorrhage of the new born. Epistaxis was fatal in one case of hereditary syphilis at the age of three years.

There were six deaths from thyrotoxicosis.

Second and third degree burns accounted for death in five patients. Here it is interesting to note that a severe active myocarditis was found in two cases, while in only one could definite evidence of renal damage be demonstrated.

There were nine cases of ulcerative colitis, five of which were associated with hemorrhage.

A survey of fifty consecutive cases of cardiac hydrothorax revealed the unexpected information that fluid was found on both sides in thirty-seven cases, on the left side in nine cases and on the right side in only four. In the majority of the cases of bilateral hydrothorax it was definitely stated that the greatest amount of fluid was found on the left side. This is in direct contradiction to the usual text book information on this point.

There were four cases of Addison's disease, three of which were tuberculous and in two of these an old tuberculous focus could only be found in the lungs. The fourth case was one of amyloid disease involving not only the adrenal but also the myocardium, lungs, spleen and liver.

In 109, or 7 per cent, the cause of death could not be satisfactorily determined. Twenty-six of these patients were senile and twenty-four were psychopathic patients, four of whom were epileptic. There were twelve infants and in six postoperative deaths no organic lesions could be found which would satisfactorily explain death. Syphilis was present in five cases and five others had a definite history of alcoholism. In three there was slight thymic enlargement but in none did a diagnosis of status thymicolymphaticus seem appropriate. In two cases there was

clinical evidence of myocardial failure but no lesion of the heart was demonstrated.

The gross and microscopic diagnosis failed to agree in ninety-five instances. In forty of these there was practically complete disagreement. It was anticipated that some valuable deductions could be made from a careful study of these discrepancies. These errors in gross diagnosis were found to be so widely distributed over such a large number of pathological conditions that very little could be salvaged from such a long and miscellaneous list. The inability accurately to diagnose cerebral syphilis without the aid of the microscope was probably the most common error. Confusing lobar pneumonia with tuberculous pneumonia and

TABLE 9

CAUSE OF DEATH UNDETERMINED

109 cases or 7 per cent

Senility	26 cases
Psychopathic	24 cases
Infants	12 cases
Postoperative	6 cases
Syphilis present	5 cases
History of alcoholism	5 cases
Enlarged thymus	3 cases
Clinical myocardial failure	2 cases

bronchopneumonia with acinar tuberculosis contributed some difficulty. Determining the correct type of non-suppurative kidney disease also seemed to be somewhat difficult. In the main, however, the gross diagnostic error was quite thinly spread over a large number of conditions.

UNUSUAL CASES

The unusual and interesting cases encountered are listed below:

Two cases of annular pancreas constituting the twenty-eighth and thirtieth cases of annular pancreas reported in the literature.

One case of spontaneous rupture of the liver due to infarction of the left lobe which was associated with suppurative pylephlebitis undetermined origin. There are apparently no cases of spontaneous rupture of the liver in the medical literature.

One patient died of rabies following a dog bite six weeks before.

Two cases of "idiopathic" dilatation of the esophagus or esophagectasia.

One case of complete absence of all genito-urinary apparatus on one side in a male. The adrenal which remained was markedly hemorrhagic (apoplexy of the adrenal).

In one case of melanoma, the liver weighed 5000 grams and was the only site of metastasis of melanoma of the retina.

In one case of generalized tuberculosis, active tuberculous infection, proved by bacterial stains, was found associated with primary carcinoma in the thyroid gland.

In a case of rheumatic heart disease with adhesive pericarditis coronary varices had developed, one of which had ruptured and a terminal hemopericardium had resulted.

There were two ruptured intracranial aneurisms involving the larger vessels (basilar and internal carotid arteries).

Rupture of an aneurism of the right common iliac artery on an arteriosclerotic basis with an unruptured aneurism of the left common iliac was found in a minister sixty-seven years of age.

There was a case of parathyroid adenoma with generalized osteitis fibrosa cystica and diffuse giant-cell tumor. In this case the patient died of uremia due to impaction of the renal pelves with insoluble calcium salts.

There was one case of paradoxical embolism with a patent foramen ovale and a saddle thrombus at the bifurcation of the abdominal aorta and one in the foramen ovale. The patient was forty-six years of age and there was an embryonal carcinoma of an undescended testis with general mestastasis.

One instance of congenital absence of a gall bladder was found.

Tuberculous endometritis was the only tuberculous lesion found in one case.

There was a case of thymoma which had metastasized to the liver, spleen, kidneys and vertebrae.

In one case a ruptured dissecting aneurism of the branch of the right anterior cerebral artery was found. There had been a severe blow on the head immediately over this area four years previously, which had not produced fracture of the skull. In the interval

the patient had had almost continual headaches, periods of dizziness, personality changes and fainted easily.

In one case hemopericardium was found due to perforation of the root of the pulmonary artery within the pericardial sac. Immediately before death, the patient, a woman, had been hit with a framed picture thrown by her husband. A narrow, finely pointed piece of glass 3 cm. in length had penetrated the chest wall to the right of the sternum. The piece of glass had recoiled into the areolar tissue of the chest wall and was found with considerable difficulty.

Multiple malignant tumors were found in two cases. In one a cerebral glioma was associated with carcinoma of the prostate. In the other case primary carcinoma of the pancreas was associated with hydronephroma of the right kidney.

Carcinoid tumor of the appendix and rectum occurred in the same individual.

SUMMARY

This analysis has been made without any attempt at strengthening or disproving any existing ideas. The findings were quite incidental and comments were made wherever they were deemed appropriate. It is obvious that no attempt has been made to account for the cause of death in all of the cases which came to autopsy. It was intended that mention be made only of those cases which might possibly be instructive or interesting.

The writer was impressed by the frequent multiplicity of diseases at the time of death, a large number of instances being encountered of the simultaneous occurrance of three or four major diseases. While this has been an interesting observation it has not been made a feature of this paper.

FOCAL CYCLIC GROWTH AS A FACTOR IN PRODUCTION OF NODULAR GOITER*

B. MARKOWITZ

From the Pathological Department, the Sloan Clinic, Bloomington, Illinois

As an introduction to the pathology of the nodular thyroid gland, some phases of its physiology should be considered, particularly the gland unit and its mode or products of secretion. The ultimate histologic unit of the gland is represented by the individual follicle composed of a sac lined by a single layer of thin, endothelial like epithelium, enclosing the colloid substance. The follicles do not branch or join others but are entirely discontinuous and are separate individual units as Rienhoff[3] has shown by a method of maceration and microdissection particularly applicable to the study of the individual follicle.

This histologic unit, as shown by Williamson and Pearse,[5] is concerned with two separate processes in the physiology of the thyroid gland:

1. The process of thyroid secretion which is active and associated with hyperplasia.
2. The process of accumulation of colloid which is passive and associated with involution.

These two processes follow each other and may be referred to as the first and second part of a functional cycle. During various periods of life either one of these two processes predominates with a resultant histological picture which is characteristic of the individual's age. The thyroid of the newly born is very active and composed of closely packed granular cells; this illustrates the process of secretion. Following the activity of infancy, the thyroid gland gradually assumes a resting stage with follicle formation and colloid retention illustrating the

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists. Philadelphia, Pennsylvania, June 7–9, 1931.

process of colloid accumulation. With the age of puberty the secretory phase of the gland again predominates sometimes so over shadowing the colloid phase that it exceeds the normal limits of activity, producing in late puberty or early adolescence what is usually termed "adolescent goiter." In later adolescence the gland again reverts to the resting or colloid stage which in adult life becomes more intensified.

These two processes occur as functional cycles in the thyroid mechanism and are the basis of the constant hyperplasia-involution process. When the body demands an increase in secretion a change in the histological picture called hyperplasia is the result and when these demands are met another histological change called involution occurs. With the latter, colloid storage with reversion to normal is seen.

When however these two processes do not follow each other in physiological order one or the other will dominate the picture with its characteristic findings. The functional cycle is then incomplete and the dominating process passes the physiological point. When this dominating process is finally counter-acted by the other, it has already passed into the abnormal state. This disproportion between these two cyclic processes at focal points is the basis of goiter nodulation as contended by Hertzler[2] and Graham.[1]

FOCAL AREAS OF INVOLUTION WITHIN A HYPERPLASTIC GOITER

The first process of physiological hyperplasia if too long continued, before the second process of involution occurs, causes the gland to become abnormally hyperplastic. A further continuation of this hyperplasia without counter-action by involution will result in a diffuse hyperplastic gland with the marked symptoms of the goiter which is clinically termed exophthalmic. At any period, usually late, during this hyperplastic state however, the first process of secretion may be arrested and followed by the second process of involution, either by medication or bodily adjustment.

With the arrest or counter-action, however, not all portions of the gland may be affected by the involution process and those

that are involved may not be acted upon to the same degree. Rienhoff[4] removed a portion of the thyroid in a small number of cases of severe Grave's disease, administered iodine for about two weeks and then removed the remaining portion of the gland. Prior to the use of iodine these glands presented the usual diffuse picture characteristic of what is clinically called exophthalmic goiter. After the use of iodine the diffuse picture was replaced by one composed of various sized, definitely circumscribed nodules. Microscopically, the change varied from a slight to a very marked degree. Even where only a slight change was noted, the dominant factor was the attempt of arrested growths with involution characterized by formation of circumscribed areas showing colloid instead of proliferating epithelium.

This involution is quite rapid when produced by the use of iodine but very slow, extending possibly over a period of years, when produced as a result of the natural exhaustion, or "burning out" process. In either case the hyperplastic cells of the follicles recede and there is formation of colloid which may later be replaced by fibrosis and regressive changes. The active process of secretion is being replaced by the passive process of involution and colloid storage. The follicles then become distended the intervening walls give way and several follicles are blended into one large colloid space. The fibrous septa surrounding this space are thickened and over a period of time forms a definitely encapsulated colloid area. The circumscribed tissue keeps going through the same uneven cycle until it becomes degenerated and with each successive step of involution the nodule becomes larger.

FOCAL AREAS OF HYPERPLASIA IN AN INVOLUTING GOITER

When the first process of secretion of a mildly hyperplastic goiter is counter-acted by the second process of involution a colloid goiter results. This colloid goiter is diffuse and smooth only when these two processes act with the same intensity over the entire gland. If, however, a single localized area of hyperplasia fails to become arrested and fails to recede to the colloid resting state, it remains as a focal hyperplastic area. Within this area there is a formation of new follicular structure with increasing

lack of orderly arrangement. Further proliferation within this localized area continues, causing it to increase in size and with increasing growth more and more pressure is exerted upon the surrounding tissue producing pressure necrosis about the periphery. In this immediate periphery atrophic changes occur and replacement fibrosis of the portions undergoing atrophy isolates this area of irregular hyperplasia with formation of the usual nodule.

EXAGGERATION OF BOTH PROCESSES

I have attempted, so far, to explain a probable factor in goiter nodule formation by showing that either the first process of secretion is overwhelmed by the second process of colloid accumulation or vice versa, that the colloid is sacrificed to secretion. There must be a third order of dysfunction of the thyroid gland in which both these processes are exaggerated with production of a temporary balanced hyperplasia. In this type the first process of hyperplasia, is not sufficiently arrested to cause a simple colloid goiter. Early in the disease however the exaggerated colloid process overcomes the secretory one at focal points. The principle involved in producing nodulation is similar to that in hyperplastic goiter or Grave's disease, except that the second process of colloid accumulation occurs early in the disease. Before the diffuse hyperplasia has had much opportunity to cause the colloid process to be sacrificed by the secretory one, the colloid accumulation occurs at focal points with production of nodules at these points.

In the thyroid gland where the first process of active secretion is counter-acted only in the later stages and at focal points by the second process of passive colloid accumulation there results focal nodulation within a diffuse hyperplastic goiter called Grave's disease, nodular exophthalmic goiter.

If the thyroid gland where the second process of passive colloid accumulation is sacrificed at focal points by the first process of active secretion there results a nodular colloid goiter, so-called non-toxic adenoma.

In the thyroid gland where there is evidence of both active and

passive disorder there results a nodular hyperplastic goiter or so-called toxic adenoma.

SUMMARY

1. There are two distinct processes in the physiology of the thyroid gland which produce a constant hyperplasia-involution cycle.

(a) Process of thyroid secretion—active with hyperplasia.

(b) Process of accumulation of colloid—passive with involution.

2. These two processes occur as functional cycles and when in disproportion produce nodulation.

(a) In diffuse hyperplastic goiter or Grave's disease, the process of secretion overwhelms the process of colloid storage. Finally, late in the disease, this colloid storage replaces the secretion at focal points with production of nodules.

(b) In nodular colloid goiter the process of colloid storage overwhelms the process of secretion. But at focal points the secretory phase continues and is not replaced by colloid storage with production of nodules.

(c) In nodular hyperplastic goiter there is over growth of both processes with colloid storage replacing thyroid secretion at focal points early in the disease with production of nodules.

REFERENCES

(1) Graham, Allen: Nodular goitres: Their relation to neoplasia. Am. Jour. Surg., **7:** 163–173. 1929.

(2) Hertzler, A. E.: Pathogenesis of goitre considered as one continuous disease process. Arch. Surg., **16:** 61–78. 1928.

(3) Rienhoff, Wm. F. Jr.: Gross and microscopic structure of the thyroid gland in man. Arch. Surg., **19:** 986–1036. 1929.

(4) Rienhoff, Wm. F. Jr.: The histological changes brought about in cases of exophthalmic goitre by the administration of iodine. Bul. John Hopkins Hosp., **37:** 285–306. 1925.

(5) Williamson, G. S. and Pearse, I. H.: The structure of the thyroid organ in man. Jour. Path. & Bacteriol., **26:** 459–469. 1923.

MODIFIED SILVER STAIN FOR TREPONEMA PALLIDUM

ELENA DE GALANTHA

Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minnesota

Since the publication of the original Levaditi method of demonstrating the presence of *Treponema pallidum* in tissues, many modifications have been suggested, designed to make the effort less laborious and more uniform in result. Hitherto, however, there has been so much "trickiness" about the application of these procedures, or it has required so much time, or the chemical adjustments have been so intricate, that the ordinary laboratory worker has found considerable difficulty in obtaining successful results with any of them.

The technic described below has so far proved to be easy of application, rapid, and uniform and positive in results:

1. Fix in formalin, 10 per cent. Tissues fixed for many years have given positive results.
2. Embed in paraffin by the usual technic and cut sections about 5 microns in thickness. Sections cut after freezing would serve as well, if cut thin enough.
3. Remove paraffin in usual manner.
4. Immerse in nitric acid, 20 per cent, for ten minutes.
5. Wash well in tap water and rinse in distilled water.
6. Immerse in silver nitrate, 3 per cent, heated to 50°C., for fifteen minutes.
7. Place slides in petri dish, sections facing up, and pour over them gently the following solution, shaking dish until sections are light brown: 5 cc. of silver nitrate, 3 per cent, to which are added 20 cc. of gelatin 3 per cent, at 40°C., and 1 cc. of hydroquinone, 1 per cent. The hydroquinone must be added quickly and the resulting mixture must be used immediately and made fresh for each batch of slides.
8. Wash slides in distilled water at 50°C. to remove gelatin; then in tap water.
9. Immerse in 1 per cent formalin for two minutes.
10. Wash in distilled water.
11. Place in hyposulphite, 2 per cent, for two minutes.
12. Dip in tap water.
13. Dehydrate, clear, and mount in Canada balsam.

EDITORIAL

Medical Research—Clinical Investigation

Fortunately, or possibly unfortunately, the English language is extremely flexible and at times burdened with words. This very attribute may lead us into the error of extreme specialization even in choosing our views, not to mention titles, for discussion. The search for nature's truths has only changed its methods, but not its modes. Human nature is still its foundation. The inquisitive one—the disbeliever—tries for new information, and youth or age is no deterrent in this quest so long as physical and mental alertness are combined with the necessary fundamental knowledge.

Fundamental investigation does not limit its fields when there are men seeking knowledge and truth. Like religion, there may be many denominations, but its value depends upon the individual affected. Two most interesting contributions have just appeared, one from the pen of a surgeon on "What Place Has Research In The Hospital"[1] and the other from a clinician on "Clinical Investigation."[2] Both are timely and worthy of serious attention and thought; both are written to promote study in the broad field of medicine—one views the subject from the standpoint of the advantages to the patient, to the hospital, to the staff and for the advancement of medicine; the other analyzes clinical investigation in the light of a constitutional pledge to a society made twenty-three years ago and reviews the changes that have intervened and the development of a maturity of thought which has already characterized other biological sciences. Especially important is the broad view taken that, argumentation over the superiority of the descriptive method, including observa-

[1] Scott, W. J. Merle: What place has research in the hospitals? Bull. Am. Hosp. Assn., **5**: 63–67. 1931.

[2] Blake, Francis G.: Clinical Investigation. Science, **74**: 27–29. 1931.

tion, analysis and deduction, as against the inductive, experimental method, or vice versa, is fruitless and narrow. Both methods are complementary halves of the whole and are, as expressed by Francis Bacon, "part of a double scale or ladder, ascendent and descendent, ascending from experiments to the invention of causes and descending from causes to the invention of new experiments." Most of us will agree with the view that disease is the inductive experiment of nature which the investigator must observe if he is to develop rational hypotheses to test by experiment.

Although personalities were not particularly noted in these articles it is evident that the qualified and alert clinical pathologist appears to occupy a happy position in the field of clinical and hospital investigation in that he is capable of viewing both the ascent and descent of the ladder from the top. If he has attained or can acquire the proper investigative or inquisitive mood he is in a position to intelligently foster this investigative attitude and promote public welfare in matters pertaining to preventing and combating human diseases.

H. J. CORPER.

SOCIETY NEWS AND NOTICES

The Eleventh Annual Convention of the American Society of Clinical Pathologists will be held in New Orleans, Louisiana, May 6–9, 1932. Dr. F. M. Johns, Chairman of the Committee on Local Arrangements, has made arrangements with the Jung Hotel to serve as headquarters for the Society. The management of the hotel has agreed to allow members of the Society to keep their reservations at the hotel throughout the meeting of the American Medical Association. Members are urged to make early reservations.

Although definite plans for the meeting have not been completed, Dr. Johns has already announced a most unusual treat. He has arranged with Dr. Denny for the Society to spend Sunday, May 8, at the National Leprosarium where a luncheon will be served and clinical demonstrations on leprosy will be given.

Dr. A. S. Giordano, Chairman of the Program and Exhibit Committee, is desirous of having titles for papers submitted to him as early as possible in order that the program may be properly prepared. No papers will be accepted for the program unless titles are submitted before April 1, 1932.

Members desiring to compete for the Ward Burdick Award must submit summaries and outlines of their papers to Dr. A. G. Foord, Pasadena Hospital, Pasadena, Calif.

The Treasurer of the Society wishes to thank the members for their prompt response to his request for the payment of dues. Names of members, whose dues are unpaid by January 10, will not be included in the mailing list of the JOURNAL.

Members of the Society are requested to notify the Secretary promptly concerning the death of any member of the Society, that the name might be passed on to the Committee on Necrology.

Dr. Dudley A. Robnett, Associate Professor of Pathology at the University of Missouri, resigned, effective September 1, after nine years as a member of the Department to accept the position of Associate Professor of Surgery of the University.

Dr. Clarence C. Pflaum, formerly Instructor in Pathology of the University of Minnesota School of Medicine, has been elected to the position of Assistant Professor of Pathology at the University of Missouri School of Medicine.

Dr. Warren T. Vaughan has recently returned from several months travel in Europe.

The Editor of the JOURNAL will represent the Society at the meeting of the Council of the Union of American Biological Societies at New Orleans on December 28th.

From time to time a commercial firm selling laboratory supplies produces a commodity of unusual value. Occasionally the JOURNAL will call attention to some of these items. No one will deny the value of the contributions of the reputable supply houses to the advancement of laboratory sciences. Such a supply house is the Arthur H. Thomas Company which has always been willing to cooperate with clinical pathologists in the development of new apparatus and chemicals Recently they have brought out the most pretentious catalog ever issued in this field. It contains 1,044 pages listing 11,814 pieces of apparatus and 2,762 reagents. This catalog is a veritable encyclopedia for which clinical pathologists should be very grateful.

BOOK REVIEWS

The Significance of Water Borne Typhoid Fever Outbreaks 1920–1930. By ABEL WOLMAN AND A. E. GORMAN. Pp. x + 82, 1931, Baltimore, The Williams & Wilkins Company, $2.00.

Those public health officials who have been accustomed to extol the effectiveness of modern methods of preserving the nation's health, will read this excellent monograph with some alarm and even dismay. In it the authors review the history of water-borne typhoid fever outbreaks during the last decade. They give data on almost 300 such outbreaks and significantly point out the fact that over two-thirds of those in the United States and three-fourths in Canada were in cities of less than 5000. By far the greater amount of water-borne illness was due to defects in collection, treatment, storage or distribution of water. Forty per cent of these outbreaks were due to these defects rather than to pollution of the raw water at its source.

The evidence clearly points to gross errors in administrative attention, while inexcusable repetitions of outbreaks occurred in five cities and in one of these from the same cause. Unprotected cross-connections between polluted fire or auxiliary water supplies and public water systems were the most important single cause of water-borne outbreaks.

The authors give the legal actions taken against administrative officials and cities in damage suits brought as a result of typhoid epidemics, making an interesting and imposing array of incidences.

The book should be read, not only by health officials and those having to do with the water supplies of our cities and towns, but by citizens interested in the health of their communities.

Pathology, Bacteriology and Applied Immunology for Nurses. By ROBERT A. KILDUFFE. Pp. xiii + 324, 1931, New York,

Milwaukee and Chicago, The Bruce Publishing Company, $2.50.

This book is intended to give the nurse an introduction to the fields of pathology and bacteriology which although elementary is a little more extensive than is usually offered in this type of book. The first part which is well illustrated and well organized deals with pathology. In this section however, there is a chapter on animal parasites which needs to be rewritten to avoid a number of errors in names and facts. At best the nomenclature in this field is difficult but the misspelled and incorrect names that occur in the text will be confusing to the student. In the second part dealing with bacteriology the author again encountered difficulty with nomenclature, for example, in the chapter on Special Pathology reference is made to *Clostridium tetani*, while in the chapter on bacteriology one finds only the name *Bacillus tetani*. Nevertheless the chapter is adequate for the use of nurses. In the chapter on Applied Immunology the author discusses serums and vaccines and concludes the book with excellent chapters on the relation of the nurse to the education of the public, in particular in relation to the propaganda of anti-vivisectionists and finally concludes with a valuable chapter dealing with methods for collecting specimens for the laboratory and laboratory exercises.

A Text Book of General Bacteriology. By EDWIN O. JORDAN. Pp. 819, 1931, Philadelphia and London, The W. B. Saunders Company, $6.00.

The tenth edition of this text book will meet with general favor as have the last editions of this well known text. The author has evidently had a struggle with his conscience to permit him to use the new generic names that are fast becoming popular in bacteriology. He has accepted almost all of them even though reluctantly. One can find, however, a few places in the next where through an oversight the old names are still retained. One may question the inclusion in this type of book of the paragraph on the sterilizing process in surgical operations. Since it is not adequately covered the information given might be quite mis-

leading. Although most of the text seems to have been rewritten, none of the new and excellent cultural methods for tubercle bacilli have been added since the last edition and one finds there no discussion of the use of animals for the diagnosis of tuberculosis.

One may seriously question in this day the advisability of including a chapter on protozoa in a text book of bacteriology. If the chapter is well done as it happens to be in this text, the author must call upon some specialist in the field to write the chapter for him. This is a tacit admission of the fact that the field lies outside of the domain of the bacteriologist and since we have perfectly adequate books dealing with this phase of the subject, it would seem that the time had come for eliminating such chapters from text books devoted to organisms that belong to the plant kingdom.

None of these objections, however, are serious enough to in any way affect either the high regard with which this book is held by students of bacteriology or its enviable position among scientific texts.

The Pathogenic Streptococci. By DAVID THOMSON AND ROBERT THOMSON. Annals of the Pickett-Thomson Research Laboratory, pp. vi + 441, 1931, London, Bailliere, Tindall and Cox, and Baltimore, The Williams and Wilkins Company, $10.00.

This volume includes a monograph on the rôle of streptococci in erysipelas, another on an historical survey of researches on the rôle of streptococci in skin diseases and lastly one on the rôle of streptococci in measles. Serious students who are concerned with this group of organisms have come to await with eagerness the appearance of these monographs. They are unique in bringing together practically all of the available literature on a subject, critically analyzing it and drawing therefrom a set of conclusions. In the first monograph the authors conclude that erysipelas is caused by a hemolytic streptococcus. They conclude that the organism *Streptococcus erysipelas* (Fehleisen) is different from *Streptococcus pyogenes* basing their conclusions on laboratory chemical tests, serological tests, skin tests, passive

immunity skin tests, the blanching test and the bacteriophage test, together with certain clinical facts. The historical treatment of the rôle of streptococci in skin diseases is well done and points out the many errors that have been made by previous investigators and the many valuable contributions that have been offered. After a thorough treatment of the subject of the relation of streptococci to measles and citing their own experiments, the authors are inclined to the belief that the organism of measles has not yet been discovered but that experiments so far favor the view that it is caused by an invisible filter-passing virus which will not grow on any culture mediums suitable for ordinary bacteria. The authors review work on the prophylaxis of measles and its treatment with convalescent serum as well as epidemiological factors and the control of the disease.